EFFECTS OF NITROGEN AND NIGHT TEMPERATURE ON THE PHYSIOLOGY OF FLOWER INDUCTION IN PINEAPPLE

[*Ananas comosus* (L.) Merrill]

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ABSTRACT

Three experiments were conducted to examine the effects of nitrogen (N) and night temperature on the physiological status and susceptibility of pineapple plants [Ananas comosus (L.) Merrill] to inflorescence initiation (forcing) with ethephon. Plants with 2.05% leaf N or exposed to a 30 C night temperature had significantly lower levels of starch and total soluble sugars in the leaves at 5:00 p.m. than plants having lower leaf N or exposed to lower night temperatures. Leaf chlorophyll concentration increased linearly with increasing levels of leaf N and the two were highly correlated ($r = 0.88$).

After application of 5.0 mg ethephon, ethylene evolution from plants on the first one or two days was significantly higher from high-N plants or from plants held at a 30 C night temperature than from low-N plants or from plants held at lower night temperatures. Ethylene evolution decreased over time and reached minimum levels three to four days after ethephon application. Ethylene evolution from the plants was assumed to be mostly from breakdown of the applied ethephon. Plants with high N or exposed to a 30 C night temperature had a lower percentage forcing than plants with lower levels of N or exposed to the lower temperatures.

The interactions between night temperature (20, ambient with a mean of 22.5, and 30 C) and leaf N (1.06 or 2.64%) were also examined but few significant interactions were found. Some

interactions may have been obscured by the relatively large amount of random variability. Leaf sucrose decreased with increasing temperature but tended to increase with increasing leaf N. Glucose was absent in leaves of high-N plants but increased with decreasing temperature in low-N plants. In the top and middle one-third of the stem, fructose remained more or less constant in low N plants as temperature increased but decreased significantly with increasing temperatures in high-N plants. Percent forcing was little affected by treatment.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

After pineapple plants [Ananas comosus (L.) Merr.] attain sufficient size, natural inflorescence initiation may occur any time throughout the year under a variety of climatic, physiological and cultural conditions. In commercial practice, however, inflorescence initiation is induced (forced) by application of growth regulators (Bartholomew and Criley, 1983). Among the advantages of forcing are the regulation of the supply of fruits both for fresh consumption and for the cannery and to make more efficient use of land and labor resources.

There are many factors that influence the successful forcing and natural floral differentiation of pineapple plants. These factors include plant size (Yow, 1959; Gowing, 1961), cultivar (van Overbeek, 1946; Das, 1965), temperature (van Overbeek and Cruzado, 1948; Yow, 1959; Bourke, 1976), photoperiod (Gowing, 1961; Friend and Lydon, 1979), and carbohydrate and nitrogen status of the plant (Nightingale, 1942; Evans 1959; Bartholomew and Kadzimin, 1977). Type, quantity and time of application of the growth regulator also affect the success of forcing (Randhawa et al., 1970; Aldrich and Nakasone, 1975; Abutiate, 1977).

Ethephon (2-chloroethylphosphonic acid) is the most commonly used growth regulator for forcing the pineapple plant. Ethephon is an effective forcing agent throughout most of the year. However, during

months when the average temperature is high, forcing can be more difficult than at other times during the year (Bartholomew and Criley, 1983). At these times, a higher rate or a double application of the forcing agent can often be used to obtain uniform results.

There is considerable evidence to show that the pattern of carbon assimilation by pineapple, a plant with Crassulacean acid metabolism (CAM) is markedly affected by air temperature (Bartholomew, 1982; Neales et al., 1980; Sale and Neales, 1980). Night temperature in general, has a greater effect than day temperature. In CAM plants a night temperature of 20 C or less favors uptake of CO_2 and accumulation of acids during the night. On the other hand, night temperatures of 35 C can result in deacidification of CAM plants during the night and may result in increased CO_2 uptake during the day (Klug and Ting, 1978). Other conditions being equal, high night temperature reduces starch accumulation and dry matter content of pineapple plants (D. P. Bartholomew, personal communication).

Night temperature influences the susceptibility of pineapple plants to floral induction with ethephon. Conway (1977) obtained a higher percentage of forcing of pineapple plants with ethephon when plants were grown at a night temperature of 20 C than at night temperatures of 25 or 30 C. The physiological reason for the low susceptibility of plants held at the warmer night temperatures was not ascertained.

Nitrogen, which is required for vigorous vegetative growth and good yield and quality of fruit, can also affect the pineapple plant's susceptibility to forcing agents. Too high a nitrogen content in the

plant may stimulate vegetative growth and reduce the susceptibility of the pineapple to inflorescence initiation with ethephon. In some growing areas and in particular where naphthaleneacetic acid or its sodium salt is used as the forcing agent, it is common to withhold nitrogen applications for four to six weeks prior to the time of forcing.

There is little or no published information on the relationship between the effect of nitrogen and night temperature on the physiological status of the pineapple plants and their relationship to floral induction with ethephon. Changes in physiological status of pineapple plants maintained at different levels of nitrogen and/or maintained at different night temperatures could provide a better understanding of the roles of nitrogen and night temperature on the susceptibility of pineapple plants to forcing with ethephon.

The objectives of the research reported here were:

1. To determine the effects of nitrogen and night temperature on the physiological status of pineapple plants, focusing mainly on levels of leaf titratable acids, leaf and stem starch, and soluble sugar contents.

2. To examine the effects of nitrogen and night temperature on ethylene evolution from pineapple plants before and after forcing with ethephon.

3. To determine the effects of nitrogen and night temperature on susceptibility of the pineapple plants to forcing with ethephon.

CHAPTER II

REVIEW OF LITERATURE

RELATIONSHIP OF TEMPERATURE TO GROWTH AND FLOWERING

Pineapple plants are native to the tropics and most are cultivated between 25° north and south latitudes (Collins, 1960). Pineapples may be found growing successfully outside these latitude limits provided that seasonal temperature changes are not extreme. Pineapple plants cannot tolerate temperatures near 0 C for prolonged periods. On the other hand, high temperatures coupled with high insolation can pose a sunburn hazard to leaves and fruit (Aubert and Bartholomew, 1973, Bartholomew and Kadzimin, 1977).

Temperature has a large influence on growth and development of the pineapple plant. When pineapple is grown in cool high elevation regions in Hawaii, leaves tend to be narrower and more rigid with a sharper degree of curvature, plants were smaller, peduncles were longer in relation to plant size, and fruits were smaller with more pointed fruitlets (Collins, 1960; Connelly, 1972). Reduced growth of pineapple plants in the winter in Hawaii was primarily due to differences in temperature rather than sunlight and soil moisture (Bartholomew and Kadzimin, 1977). Day and night temperatures for optimum growth of pineapple plants had been reported to be about 30 and 20 to 22 C respectively (Neild and Boshell, 1976; Bartholomew and Kadzimin, 1977). The optimum temperatures for root and leaf elongation are about 29 and 32 C respectively (Sanford, 1962). Ravoof

(1973) measured increased growth and nutrient uptake of pineapple plants when the root temperature was increased from 20 to 30 C.

Pineapple plants possess Crassulacean acid metabolism (CAM) (Sideris et al., 1948; Seshagiri and Suryanarayanamurthy, 1957), a carbon assimilation pathway in which leaves accumulate organic acids, primarily malic acid, during the night concomitant with dark assimilation of CO_2 (Klug and Ting, 1978). CAM plants utilize these organic acids as a source of CO_2 for photosynthesis during the day (Ranson and Thomas, 1960; Dittrich et al., 1973). The intensity of CAM which is carried on by the plant has long been known to be influenced by environmental factors, especially temperature (Bennet-Clark, 1933). Bennet-Clark (1933) reported that a night temperature of 20 C or less favored dark acidification whereas temperatures of 30 C or higher enhanced dark decidification.

Temperature has also been shown to affect stomatal opening and CO_2 assimilation. Measurement of leaf diffusive resistance to water vapor transfer in pineapple leaves showed that stomata opened at night, partially opened in the early morning, and closed during the day (Neales et al., 1968; Bartholomew, 1975; Bartholomew and Kadzimin, 1976; Bartholomew, 1982). However, on cool or cloudy days the stomata can be partially open (Aubert, 1971). The difference between day and night temperature during one day has been reported to influence the opening and closing of stomata on the subsequent day (Yoder, 1969; Connelly, 1972). Cooler temperature, either during the light or dark period favored opening of the stomata (Neales et al., 1980). The pattern of stomatal opening may influence the movement of forcing

agents into the plant (Bartholomew and Kadzimin, 1977; Aldrich and Nakasone, 1975).

At a constant day temperature of 35 C, dark CO_2 fixation and presumably malic acid synthesis by pineapple increased as night temperatures decreased from 35 to 20 C (Connelly, 1972). A night temperature of 35 C induced a small net efflux of CO_2 during the night and a large net influx of CO_2 on the following day (Neales, 1973). Total CO_2 assimilation during a 24-hour light/dark cycle also has been shown to decrease with increasing night temperature. At a day temperature of 30 C, increasing night temperatures from 20 to 35 C reduced total assimilation of CO_2 from 6.5 to 1.3 g CO_2 m^{-2}(leaf area) day^{-1} (Neales et at., 1980). The proportion of CO_2 assimilated at night was also reduced from 90% to 40%.

Day temperatures also have some influence on total CO_2 assimilation during the 24-hour light/dark period. At a constant night temperature of 15 C, decreasing day temperatures from 25 to 10 C decreased total CO_2 assimilation per 24-hour period from 9.3 g CO_2 m^{-2} day^{-1} to 3.6 g CO_2 m^{-2} day^{-1} (Neales et al., 1980). However, at a night temperature of 25 C, day temperatures over the range from 10 to 30 C had little or no effect on total CO_2 assimilation which remained approximately constant at 3.0 g CO_2 m^{-2} day^{-1}. Pineapple plants exposed to thermoperiods having a small diurnal variation may take up CO_2 at similar rates during the day and night (Connelly, 1972). Thermoperiods with large diurnal temperature changes cause proportionally more CO_2 assimilation during the night (Connelly, 1972).

Temperature can also enhance or retard the change from the production of vegetative structures at the growing point to the production of reproductive structures. Low temperature promotes inflorescence initiation in many plants. Thompson (1944) grew celery at low (5-11 C) and medium temperatures (15-20 C) for 10, 20 and 30 days and found that low temperature initiated flowering and the percentage of flowering increased as duration of exposure to low temperature was increased. A similar response has been observed for many other species (Wareing and Phillips, 1978). Wareing and Phillips (1978) also mentioned that most plants which have a rosette habit of vegetative growth and show marked internode elongation in the flowering phase will have flower initiation associated with low temperature. Pineapple, to some degree, has vegetative growth which could be characterized as a semi-rosette type because the internode length between leaves is very short. The flowering phase of pineapple plants is associated with rather marked elongation of the peduncle. There are few data available about the relationship between low temperature and flowering in pineapple. Exposure of plants to a 15 C temperature for a single night or several nights of moderately low temperature (about 18 C) induced differentiation of floral primodia in field-grown pineapple (Nightingale, 1942). van Overbeek (1946) reported that a winter temperature of 15 to 16 C in Puerto Rico induced uniform floral differentiation in 'Red Spanish' pineapple plants; however, the Cabezona cultivar did not flower uniformly in response to the same low temperature. In Papua New Guinea, floral initiation of rough leaf pineapple was believed to be associated with

minimum temperatures of 20 to 23 C which occurred seasonally in that area rather than to sunshine duration or moisture availability (Bourke, 1976).

Yow (1959) reported a positive correlation between floral differentiation in pineapple and a low temperature (5 C) which persisted for two to three days in January in Taiwan; however, he stated that other factors such as time of planting, planting density, and fertilizers also play important roles. Gowing (1961) studied the effect of photoperiod and low temperature on flowering of 'Smooth Cayenne' pineapple in Hawaii. He noted that short days had a greater influence on flowering than night temperature because plants exposed to cool night temperatures which were given a light break to maintain physiologically long day conditions did not flower. He concluded that 'Smooth Cayenne' was a quantitative but not an obligate short-day plant. However, at the same daylength, low night temperature resulted in earlier flowering.

Friend and Lydon (1979) examined the effect of photoperiods of 8, 10, 12, and 16 hours on the flowering behavior of pineapple plants cv. Smooth Cayenne maintained at a constant day and night temperature of 25 $\pm$ 2 C. Results of the work agree with those of Gowing (1961) that photoperiod significantly affected flowering of 'Smooth Cayenne' pineapple. Under an eight-hour daylength, 90% of the plants had flowered after 692 days. Only 8% of the plants under a 12-hour daylength had flowered and plants grown under a 16-hour daylength were vegetative. After 900 days of growth, the flowering of plants was 100, 69, 53, and 30% under 8, 10, 12, and 16-hour daylengths,

respectively. They concluded that flowering of 'Smooth Cayenne' pineapple was controlled by daylength and promotion of flowering did not require either diurnal variation in temperature or a temperature below 25 C. However, under a natural daylength of approximately 11 hours, pineapple plants grown in the field flowered approximately 500 days after planting (Bartholomew and Kadzimin, 1977). Friend and Lydon (1979) suggested that differences in growing conditions and other environmental factors may have been responsible for the low percentage of flowering obtained in 12-hour days.

In a later experiment, Friend (1981) found that pineapple plants maintained at a daylength of eight hours and a night temperature of 20 C flowered 470 days after transfer to the night temperature treatment. The mean number of days to flowering increased to 610 and 660 days for plants grown at 25 and 15 C respectively. Plants grown at a 30 C night temperature were still vegetative after 690 days. Friend (1981) concluded that pineapple is not a typical short day plant since both short daylength and low night temperature significantly accelerated flowering.

NITROGEN STATUS AND FLOWERING IN PINEAPPLE

Nitrogen is necessary for the growth of pineapple plants as it is an important constituent of proteins, nucleic acids and other nitrogenous compounds. These constituents account for about 7% of pineapple dry matter (Nightingale, 1942). Yields are determined by plant size and number of plants producing fruit (Evans, 1957). In order to obtain fruit of adequate size, the mother plant should be

supplied with an adequate amount of nitrogen at the time of floral differentiation (Nightingale (1942). However, high nitrogen levels in the plant cause vigorous vegetative growth which may reduce susceptibility of the plant to floral initiation either naturally or with chemical agents (Nightingale, 1942; Bondad, 1973; Aldrich and Nakasone, 1975).

Nightingale (1942) believed that low nitrate reserves together with low temperature influenced the time of floral differentiation in pineapple plants. An empirical formula was employed to estimate the date of floral differentation by using the percentage of leaf nitrate-nitrogen multiplied by the minimum temperature on any given night. When the value of this empirical differentiation index reached about 3.00 or less, the first stage of differentiation was found about five days later.

The level of nitrogen that influences the flowering behavior of pineapple plants may be related to the level of carbohydrate reserves in the stem or leaf, or both. High levels of carbohydrate relative to nitrogen usually favor flowering. Nightingale (1942) reported that pineapple plants which had a high carbohydrate/nitrogen ratio generally flowered earlier than those which did not.

High levels of leaf nitrogen may also reduce susceptibility of pineapple plants to chemical forcing (Bartholomew and Kadzimin, 1977). Py and Guyot (1970) and Aldrich and Nakasone (1975) reported that pineapple plants which had a higher nitrogen content in the leaves at the time forcing agents were applied required more growth regulator to

achieve nearly 100% flowering than plants with low levels of nitrogen. Plants that received fertilizer one month before a single application of calcium carbide had a much lower percentage of flowering than unfertilized plants (Aldrich and Nakasone, 1975). A double application of the growth regulator increased flower initiation to over 90%. However, no further increase in flowering percentage was obtained with three applications. Rapid vegetative growth as a result of high nitrogen fertilizer and good water availability reduced the percentage of plants which were forced (Evans, 1959; Wee and Ng, 1968; Py and Guyot, 1970). Bartholomew and Kadzimin (1977) mentioned that the environmental factors which promoted flowering were those which retarded vegetative growth. Those factors included reduced temperature and solar radiation and a decrease in nutrition and water supply. However, excessive stress may also reduce plant susceptibility to the forcing agent (D. P. Bartholomew, 1981, personal communication).

INFLORESCENCE INDUCTION IN PINEAPPLE WITH GROWTH REGULATORS

Artificial induction of flowering in pineapple plants by the application of growth regulators has been widely practiced for a long time. During the late eighteenth century, pineapple growers in the Azores Islands induced flowering of pineapple plants by exposing them to smoke (Collins, 1960). Research by Rodriquez (1932) led to the identification of ethylene as the active ingredient in smoke. It was later found that a wide variety of growth regulators could be used to

induce pineapple plants to flower. Those chemicals included acetylene (Kerns, 1936; Lewcock, 1937; Das et al., 1965), naphthaleneacetic acid (NAA), naphthaleneacetamide (NAD) (Clark and Kerns, 1942; Randhawa et al., 1970), 2,4-dichlorophenoxyacetic acid (2,4-D) (van Overbeek, 1946; Evans, 1959), and beta-hydroxyethylhydrazine (BOH) (Gowing and Leeper, 1955; Norman 1977; Chew and Malek, 1978). More recently, the growth regulator ethephon was found to induce early and uniform flowering in pineapple (Cooke and Randall, 1968; Wee and Ng, 1971; Dass et al., 1976).

Acetylene, generated upon hydrolysis of calcium carbide, is used on smaller fields in many pineapple growing areas in less developed countries due to its relatively low cost and ready availability in local areas. In Queensland, Lewcock (1937) reported that acetylene can be used to force pineapple plants into flower at almost any stage of growth provided that plants are well established and climatic conditions are favorable at the time the treatment is applied. For 'Sugarloaf' pineapple in Ghana, Norman (1975, 1977) indicated that calcium carbide was as effective a forcing agent as BOH or ethephon. The most effective method is to apply granulated calcium carbide or a solution of acetylene to the center of the plant (Lewcock, 1937; Teisson, 1979). The rate of application of dry calcium carbide ranges from 150 mg (Aldrich and Nakasone, 1975) to 3.0 g per plant (Norman, 1977).

Naphthalene acetic acid (NAA) and related compounds can also induce flowering in pineapple plants. Clark and Kerns (1942) first

reported that floral initiation in pineapple could be obtained by application of alpha-naphthalene acetic acid, naphthaleneacetamide or naphthalenethioacetamide. In Puerto Rico, NAA and 2,4-D at the rate of 0.25 to 0.50 mg per plant were equally effective in inducing flowering in 'Cabezona' pineapple (van Overbeek, 1946). However, fruits produced from plants treated with 2,4-D were smaller than fruits from NAA treated plants.

Sunlight causes destruction of NAA, especially when the temperature is high (Gowing et al., 1962). Despite this fact, day application of NAA has proven to be the most effective.

NAA is absorbed and translocated very quickly especially from the younger leaves, to the apical meristem (Gowing et al., 1962) so any rain that follows its application does not significantly reduce its effectiveness (Py and Tisseau, 1965). However, in warm climates with frequent rains, a second application of NAA seven to eight days after the first one or a higher rate may be required to obtain a high forcing percentage (Groszmann, 1950; Py and Guyot, 1970).

Beta-hydroxyethylhydrazine (BOH) has also been shown to be an effective forcing agent for pineapple plants (Gowing and Leeper, 1955; Cibes and Gandia, 1962; Py and Guyot, 1970; Norman, 1975, 1977). Cibes and Gandia (1962) obtained 100% flowering of 'Red Spanish' pineapple by application of BOH at the rate of 30 to 60 mg per plant either directly to the center of the plants or by a spray over the whole plant. Flowering was more uniform and rapid than with calcium carbide treated plants. However, Py and Guyot (1970) reported that

application of 0.1 g BOH per plant to the center of the plant was more effective than spraying over the whole plant.

Ethephon, a relatively new plant growth regulator, decomposes at a pH above 4.0 to yield ethylene, Cl^-, and phosphate (Cooke and Randall, 1968). Maynard and Swan (1963) suggested that the mechanism of ethylene production from ethephon involves the nucleophilic attack on the phosphonate dianion by a water molecule and elimination of chlorine leading to the formation of ethylene, phosphate and chloride ions. The mechanism was later confimed by Yang (1969). When ethephon was applied to plant tissue, it was degraded with liberation of ethylene at the cytoplasmic pH of the plant cell, causing responses characteristic of ethylene treatment (Cooke and Randall, 1968; Yang 1969).

Ethephon is rapidly gaining popularity in physiology and agriculture due to its being a convenient way to apply ethylene treatments to plant tissues (De Wilde, 1971; Bondad, 1976). It is also an effective flower inducing agent for many cultivars of pineapple including Smooth Cayenne (Cooke and Randall, 1968), Kew (Randhawa et al., 1970), Singapore Spanish (Wee and Ng, 1971), Sugarloaf (Norman, 1975), and Masmerah (Wee and Rao, 1979). Urea is generally added as an adjuvant at concentrations ranging from 2 to 5% (Dass et al., 1975; Glennie, 1980). Urea is thought to enhance absorption of ethephon into plant tissue (Yamada et al., 1965) and also increases the rate of ethephon breakdown by increasing the pH of the solution (Warner and Leopold, 1969; Aubert, 1973; Dass et al., 1975).

In addition to those already mentioned, a number of factors influence susceptibility of pineapple plants to chemical forcing. Within certain limits, large plants were found to be more susceptible than small plants (Cooper, 1942; Das et al., 1965; Bondad, 1973; Conway, 1977). Bondad (1973) reported that five to eight-month old plants flowered readily after application of 50 mg ethephon. Younger plants (three to four months old) flowered but inflorescence initiation was delayed relative to eight-month old plants while one to two- month old plants did not respond to the same forcing treatment. Conway (1977) reported a higher percentage of flowering of six and eight month old pineapple plants (mean dry weight 206 and 342 grams respectively) than of four month old plants (mean dry weight 114 grams) treated with ethephon. No inflorescences were formed on two month old plants (mean dry weight 65 grams) treated with ethephon but the plant stem became longer and more tapered. However, when plants are very large and are growing vigorously, they may be more difficult to force or may need more chemical to obtain the same percentage of flowering (Py and Guyot, 1970; Aldrich and Nakasone, 1975).

Temperature affects the susceptibility of pineapple plants to forcing agents. Bartholomew and Kadzimin (1977) reported that seven and nine month old pineapple plants grown at night temperatures of 18 and 22 C could be forced with ethephon while plants grown at 26 and 30 C could not. Conway (1977) exposed two, four, six and eight month old pineapple plants to night temperatures of 20, 25 and 30 C for 7, 14, and 21 days. As was mentioned previously, the two month old plants

were too small to be forced with ethephon. The four month old plants initiated flowers in response to ethephon especially at the 20 C night temperature and higher percentages of flowering were obtained with six and eight month old plants. Night temperatures of 25 and 30 C significantly decreased the percentage of flowering.

The time of day that growth regulators are applied also influences the effectiveness, or the susceptibility of the pineapple plants to some forcing agents. A higher percentage of flower induction has been obtained from night or early morning application of acetylene and ethylene than during the day (Aldrich and Nakasone, 1975; Abutiate, 1977). Bartholomew and Kadzimin (1977) suggested that this phenomenon was due to the inverted stomatal rhythm of pineapple which has Crassulacean acid metabolism, thus the stomata are open primarily at night.

Chemicals vary in their effectiveness as forcing agents. Ethylene and related compounds are better forcing agents than NAA and other auxin compounds. Randhawa et al., (1970) compared the effectiveness of Ethrel (ethephon), NAA, and NAD as forcing agents on the Kew variety of pineapple. Flower initiation of Ethrel treated plants was very uniform while flower initiation continued over a longer period for NAA and NAD treated plants. In Malaysia, ethephon at the rate of 10 to 40 mg per plant was also found to be superior to acetylene or SNA (sodium salt of naphthaleneacetic acid) (Wee and Ng, 1971). Wee and Rao (1979) studied the cytohistological changes within the stem apex of the 'Masmerah' pineapple after flower induction with

acetylene, NAA, and ethephon. Plants forced with acetylene and NAA initially had a more pointed apex, while plants forced with ethephon produced a broader apex. Fruit produced from plants forced with ethylene and related compounds forms a more or less uniform cylinder while those forced with NAA and related compounds produced a more tapered cylinder. The use of ethylene and related compounds also resulted in more uniform fruit ripening and a narrower harvesting peak than NAA and related compounds (Terry, 1975).

Burg and Burg (1966) demonstrated that application of NAA to pineapple plants resulted in ethylene production by the plant. Ethylene production peaked about five days after application of the NAA. Burg and Burg (1966) concluded that ethylene produced as a result of NAA application was responsible for flower induction of the pineapple plants. It was later confirmed that auxins stimulate ethylene production in many plant tissues (Abeles, 1973; Yu and Yang, 1979; Yu et al., 1979).

ETHYLENE BIOSYNTHESIS FROM HIGHER PLANTS

Considerable evidence has accumulated in the past two decades to show that ethylene production in tissues of higher plants derives from methionine (Lieberman and Mapson, 1964; Abeles, 1973; Yang, 1980). The recent discovery of Adams and Yang (1977) that S-adenosyl-methionine (SAM) is an intermediate between methionine and ethylene led to their further discovery that the amino acid, 1-aminocyclopropane-1-carboxylic acid (ACC) is the immediate precursor

of ethylene. Adams and Yang (1979) have established that the sequence of metabolites in the ethylene biosynthetic pathway are as follows: Methionine ---> SAM ---> ACC ---> ethylene. In the conversion, carbon one of methionine is converted to CO_2, carbon two to formic acid, carbon three and four to ethylene (Burg and Clagett, 1967; Yang, 1974). It has been confirmed that this pathway operates in auxin induced ethylene production in vegetative tissue (Yu and Yang, 1979) and when ethylene is produced by plants under stress (Yu and Yang, 1980).

Production of ethylene is known to be regulated by various physiological and environmental factors. As part of the normal life of a plant, ethylene production is induced during certain stages of growth such as germination, ripening of fruit, and the abscission of leaves, flowers and fruits (Kende and Baumgarter, 1974; Lieberman, 1979; Yang, 1983). Factors that affect ethylene production include temperature and various kinds of stress such as wounding and water stress (Yang, 1980). Growth regulators such as auxins, cytokinin, and abscisic acid (ABA), also affect the synthesis and action of ethylene (Lieberman, 1979; Yang, 1980).

Generally the optimum temperature for ethylene production in plant tissues ranges from about 25 to 30 C (Eaks, 1978; Burg, 1962; Yu et al., 1980). As temperature increases above 30 C the rate of production falls until it ceases at about 40 C (Burg, 1962). Yu et al. (1980) examined the effects of temperature on ethylene production and on the levels of metabolic intermediates in plugs of apple tissue

and in mungbean hypocotyls incubated at temperatures ranging from 25 to 40 C. The optimum temperature for ethylene production for both tissues was 30 C. A temperature higher than 30 C resulted in a decline in the rate of ethylene production and only trace amounts of ethylene were produced from tissues incubated at 40 C. However, the level of ACC in apple tissue increased progressively as temperature was increased from 25 to 40 C. Yu et al., (1980) concluded that both ACC synthesis and conversion to ethylene increased as temperature increased from 25 to 30 C. As temperature increased from 30 to 40 C ethylene production decreased but the ACC content of the tissue increased, indicating that conversion of ACC to ethylene was the primary site of temperature inhibition.

Auxins are known to stimulate ethylene production in a wide variety of plant tissues (Abeles, 1973; Burg and Burg, 1966; Lieberman, 1979). Lieberman and Kunishi (1975) showed that auxin-induced ethylene production in subapical sections of pea seedlings occurred after a lag period of about one to three hours. The continuous presence of auxin was required for ethylene production and ethylene synthesis was inhibited by inhibitors of RNA and protein synthesis. They suggested that ethylene synthesis was induced by a high level of auxin and may involve RNA and protein synthesis.

Yu and Yang (1979) and Yu et al. (1979) examined the mechanism of auxin induced ethylene synthesis using mungbean hypocotyls. They reported that IAA stimulated ethylene production 500 times over that of the control. Active conversion of methionine to ethylene was

observed only in the presence of auxin. However, in the absence of auxin, the tissue could convert methionine to SAM and ACC to ethylene but could not convert SAM to ACC. The data indicated that auxin exerted its regulatory function on a step involved in the conversion of SAM to ACC. They concluded that auxin stimulated ethylene production by inducing the synthesis of the enzyme ACC synthase, which catalyzes the conversion of SAM to ACC.

Since ethylene plays an important role in floral induction of pineapple, factors that influence ethylene synthesis and action may also affect susceptibility of the plants to forcing treatment.

CHAPTER III

MATERIALS AND METHODS

PLANTING MATERIAL

The tops of fruit (crowns) of 'Smooth Cayenne' pineapple having uniform weight were used as planting material for all of the following experiments. The crowns were dipped in a fungicide solution (Difolatan 14.4 grams per liter of water), inverted and dried for seven days. After curing the crowns were planted 2.5 cm deep in a 1:1 by volume cinder-vermiculite medium in 15 cm diameter plastic pots. Cinder retained on a 3.2 mm (1/8 inch) mesh was used. The crowns were watered daily with tap water for one week. The plants were kept in the greenhouse under the ambient temperature and photoperiod. In the ensuing period, a stock nutrient solution, prepared by dissolving 3.40 g of a complete fertilizer (Gaviota foliar 62) (Table 1), supplemented with 1.25 g of $CaCl_2.2H_2O$, 3.44 g of $MgSO_4.7H_2O$, 0.51 g of Sequestrene iron chelate (Ciba-Geigy Fe 330) and 2.64 g of urea in one liter of water, was applied to the plants three times a week through a siphoning proportioner which mixed approximately one part of stock solution with 16 parts water giving the composition and concentration of nutrients shown in Table 2. Diazinon insecticide was sprayed when necessary to control scale insects.

EXPERIMENT I.

Five months after planting, the plants were transferred to 25 cm diameter plastic pots and additional fresh media was packed around the

Table 1. Analysis of the fertilizer Gaviota Foliar 62.

Gaviota Foliar 62	Analysis
	- % -
Ammonium nitrogen	10.0
Nitrate nitrogen	2.5
Phosphoric acid	25.0
Water soluble potassium	20.0
Magnesium	0.04
Boron	0.01
Molybdenum	0.02
Iron	0.10
Manganese	0.012
Copper	0.013
Zinc	0.014

Table 2. Composition of nutrient solution utilized for the culture of pineapple.

Nutrient	Final concentration
	- ppm -
N	96.58
P	15.80
K	50.00
Ca	20.08
Mg	20.08
S	26.31
Cl	35.51
Fe	3.20
B	0.020
Cu	0.026
Mn	0.024
Mo	0.040
Zn	0.028

side of the pots. The plants were grown as described above for one additional month.

To establish the experiment, the plants were grouped into classes based on the length of the longest leaf ('D-leaf'). The D-leaf is the youngest physiologically mature leaf (Sideris et al., 1938; Krauss, 1948). Plants having approximately the same D-leaf length were grouped into three replicates. The treatments consisted of a factorial arrangement of four levels of nitrogen and two levels of ethephon with eight plants per treatment arranged in a randomized complete block design. In order to avoid confounding the effects of nitrogen and plant size on forcing, the nitrogen treatments were not begun until the plants were six months old.

The levels of nitrogen in the plant were established by varying the amounts of urea added to the nutrient solution. The final concentrations of nitrogen in the culture solution were 25, 60, 96, and 168 ppm for treatments N_1, N_2, N_3, and N_4 respectively. However, leaf nitrogen levels four weeks after the start of treatment ranged from only 0.8 to 1.2%, a range considered to be too narrow to permit evaluation of the effect of plant nitrogen content on forcing. To extend the range of nitrogen levels in the leaves, the plants were sprayed weekly to runoff for four additional weeks with solutions containing 0.5, 1.0, 2.0, and 4.0% urea for treatments N_1, N_2, N_3, and N_4 respectively.

On February 7, 1982, the day of forcing which was three days after the last application of fertilizer solution, three D-leaf samples were harvested from plants from each level of nitrogen in

each replicate at 7:00 a.m. for the measurement of leaf titratable acids, nitrogen, and chlorophyll. Two plants from each level of nitrogen in each replicate were harvested at 5:00 p.m. for the measurement of total fresh and dry weight of leaves and stem and stem starch and total soluble sugars. Three D-leaf samples were also harvested from plants from each level of nitrogen in each replicate at 5:00 p.m. for the measurement of leaf starch, total soluble sugars and titratable acid. Inflorescence initiation was accomplished by pouring 20 ml of 4% urea-water solution containing 0.5 or 5.0 mg ethephon into the center of each plant at 6:00 p.m.

Ethylene evolution from one plant from each treatment in each replicate was measured by enclosing each plant in a 30 liter vinyl plastic chamber during the two nights before and five nights after ethephon application. Some leaves were trimmed from each plant on the day of measurement to facilitate sealing of the plant into the chamber.

Thirty eight days after forcing, the plants were harvested and the percentage of forcing was determined after dissection of the growing point. Length and diameter of the developing inflorescence and peduncle were measured and number of florets per inflorescence were estimated by counting the number of florets on one long spiral (Bartholomew, 1977) and multiplying by eight.

EXPERIMENT II.

The plants for this experiment were grown as described previously. After seven months growth the plants were arranged into

a 3x3 factorial experiment with three replications. The treatments were night temperatures of 20, 25, and 30 ± 2 C and durations of exposure prior to forcing of 21, 14 and 7 days. Treatments were initiated so forcing for all three durations could be done on the same day. There were five plants per treatment. Since space in the temperature controlled chambers was limited, some replications were done separately.

For each replication, plants of approximately the same size selected on the basis of the length of the D-leaf, were put into the chambers. The day temperature for all chambers was maintained at 30 ± 2 C.

Ethylene evolution from one plant from each treatment within each replicate was measured by enclosing the plant in a vinyl plastic chamber overnight two days before and four days after ethephon application. The plants were held at the treatment temperature during the night prior to collection of the sample for ethylene measurement.

Forcing was done on May 23, 1982 for replications one and two and on June 27, 1982 for replication three. Ethephon at the rate of 5 mg in 20 ml of 4% urea solution was applied by pouring the solution into the center of the plants at 6:00 p.m. The plants were left in the temperature controlled chamber for two more weeks to avoid possible effects of temperature changes on inflorescence development of the plants. On the day of forcing, leaf samples (D-leaves) were collected from three and four plants respectively from each treatment

within each replicate at 7:00 a.m. for the measurement of titratable acids and nitrogen. Leaf starch and total soluble sugars, and titratable acids were also measured in D-leaf samples collected from three plants from each treatment within each replicate at 5:00 p.m. One plant from each treatment in each replicate was collected for the measurement of fresh and dry weight of leaves and stem and starch and total soluble sugars in the top and middle thirds of the stem.

At 39 days after forcing, the plants were harvested and the percentage of forcing and inflorescence growth was determined according to the method described previously.

EXPERIMENT III.

The crowns for Experiment III were treated and planted in the greenhouse as was described previously for a period of two months. Due to a slow growth rate, which probably was caused by nutrient toxicity (W. G. Sanford, personal communication), the nutrient solution was changed to that developed by Sanford (W. G. Sanford) and modified by Conway (1977). The plants and planting medium were flushed frequently with tap water. A concentrated stock solution was prepared and applied to the plants through a siphoning proportioner which mixed approximately one part of stock solution with 16 parts of water to provide the composition and concentration of nutrients shown in Table 3.

After five and a half months growth, the plants were arranged into a 3x2 factorial experiment. There were three replications with

Table 3. Stock solution and final concentration (after 1:16 dilution) of nutrient solution utilized for the culture of pineapple.

Nutrient source	Stock solution	Nutrient	Final concentration
	- (g l^{-1}) -		- ppm -
Ammonium Nitrate (NH_4NO_3)	3.3980	N	95.00
Magnesium Nitrate ($MgNO_3.6H_2O$)	3.5861	P	15.50
Potassium phosphate (KH_2PO_4)	1.1544	K	58.50
Potassium Sulphate (K_2SO_4)	1.4779	Ca	20.00
Calcium Chloride ($CaCl_2.2H_2O$)	1.2272	Mg	20.00
Boric Acid (H_3BO_4)	0.0122	S	16.00
Magnesium Chloride ($MgCl_2.H_2O$)	0.0075	Cl	35.00
Zinc Sulphate ($ZnSO_4.7H_2O$)	0.0010	Fe	5.00
Copper Sulphate ($CuSO_4.5H_2O$)	0.0003	B	0.10
Molybdic Acid ($H_2MoO_4.H_2O$)	0.0001	Cu	0.005
Sequestrene Iron Chelate (Fe 330)	0.8503	Mo	0.003
		Zn	0.013

six plants per treatment. The treatments were night temperatures of 20 and 30 ± 2 C and the ambient greenhouse temperature, and low and high nitrogen. The plants were selected for each replication on the basis of the length of the D-leaf. Differential nitrogen levels were established six weeks prior to the temperature treatments. Low nitrogen plants (N_1) continued receiving the nutrient solution shown in Table 3. High nitrogen plants (N_2) were established by spraying to runoff a solution containing 2% urea on the plants at six day intervals for six weeks.

After an additional one and one-half months growth, a total of seven months growth, the plants were placed in temperature controlled chambers with the day temperature maintained at 30 ± 2 C. The plants in the ambient temperature were left in the greenhouse at an average day temperature of 28.5 C and an average night temperature of 22.5 C. The plants were exposed to the night temperature treatments for two weeks before forcing with ethephon.

Ethylene evolution from one plant from each treatment within each replicate was measured by enclosing the plant in a plastic chamber for one night one day before and for four days after ethephon application. Trimming of some leaves to facilitate sealing of the plant into the chamber was done three days before the measurement.

On the day of forcing, D-leaf samples for titrable acids and chlorophyll were collected from four plants from each treatment in each replicate at 7:00 a.m. Leaf titratable acids were measured again at 5:00 p.m. Leaf nitrogen, starch, total soluble sugars and sucrose, glucose and fructose were measured in the leaf samples

collected from five plants from each treatment in each replicate at 5:00 p.m. One plant from each treatment was collected at random from each treatment in each replicate for the measurement of fresh and dry weight of leaves and stem and starch and sugars in the top and middle part of the stem. Forcing was done on May 9, 1983 for replication one and on May 30, 1983 for replications two and three. Ethephon at the rate of 5 mg in 20 ml of 4% urea solution was applied by pouring the solution into the center of the plants at 6:00 p.m. The plants were left in the temperature controlled chambers at the treatment temperatures for one more week to avoid possible effects of temperature changes on inflorescence development of the plants.

At 36 days after forcing, the plants were harvested and the percentage forcing and inflorescence development were determined as previously described.

ANALYTICAL METHODS

Determination of Leaf Titratable Acidity

Titratable acidity in leaf tissue was determined following the procedure of Sideris et al. (1948) as modified by Moradshahi et al., (1977). About 10 g from the middle one-third of the D-leaf was cut into small pieces and blended with 45 ml of distilled water for about three minutes. The homogenate was transferred into 250 ml beakers and boiled for about three minutes. After cooling, the mixture was filtered through Whatman #1 filter paper and brought to 100 ml with distilled water. After centrifugation at 4000 rpm for about 10 minutes, a 30 ml aliquot was titrated with 0.05 N NaOH to pH 8.3.

The amount of acids present, expressed as milliequivalents of acid per 100 g fresh weight of leaf was calculated from the volume of NaOH used in the titration.

Determination of Chlorophyll

Chlorophyll in the leaf tissue was determined by the procedure of MacKinney (1941) and Arnon (1949). About 5 grams of tissue taken from middle one-third of the D-leaf was cut into small pieces and homogenized with 40 ml 80% acetone and 20 mg $MgCO_3$ for three minutes. The homogenate was filtered twice through Whatman #1 filter paper and brought to 100 ml with 80% acetone. The optical density (D) of the extracts was determined with a Bausch and Lomb Spectronic-100 spectrophotometer at wavelengths of 663 and 645 nm. Total chlorophyll in milligrams per gram fresh weight was calculated according to the equation of MacKinney (1941) as follows:

$$C = 20.2\ D_{645} + 8.02\ D_{663}$$

Where C = total concentration of chlorophyll

D_{645} = absorption at 645 nm.

D_{663} = absorption at 663 nm.

Determination of Total Nitrogen in Leaf Tissue

Total nitrogen in pineapple leaf tissue was determined by the procedure of Mitchell (1972). A portion of the middle one-third of the D-leaf from each treatment was oven dried to a constant weight at

70 C. About 0.05 g of the leaf sample ground to 20 mesh and three boiling chips were placed in a 75 ml digestion tube. One g of mixed salts, 20:0.5:2 g of K_2SO_4:$FeSO_4$:$CuSO_4$, was added to the tube followed by 1.0 ml of H_2O_2 and 2.0 ml of concentrated H_2SO_4. After mixing, the samples were predigested at 150 C for 30 minutes. The samples were then digested at 375 C for three hours, cooled, diluted to 75 ml with deionized water and mixed well. The concentration of ammonium ions in the digested solution was determined according to the method of Mitchell (1972). A 2.0 ml aliquot was transferred into a 100 ml volumetric flask followed by 1.0 ml of 1% EDTA (pH 10). Five ml of solution A (6.24 g NaOH in 1000 ml H_2O), 10.0 ml of solution B (5.0 g phenol and 25 mg sodium nitroprusside in 500 ml H_2O), and 10.0 ml of solution C (2.5 g NaOH, 1.87 g Na_2HPO_4, 15.9 g $Na_3PO_4.12H_2O$, and 5.0 ml 5.25% sodium hypochlorite in 500 ml H_2O) were added and the solution was brought to 100 ml volume with deionized water. After standing for one hour, the absorbance was measured at 625 nm. The amount of nitrogen in the tissue on a percent dry weight basis was calculated from a standard curve.

The blank was prepared by digesting 50 mg of filter paper and treating it the same way as the sample. A standard curve was prepared by dissolving 0.472 g of $(NH_4)_2SO_4$ in 1000 ml deionized water. Appropriate aliquots were taken from the stock solution and transferred to the filter paper digests and treated in the same manner as the unknown to give working standards ranging from 10 to 50 ppm N.

Determination of Starch Content

Pineapple D-leaf and stem tissue was oven dried to a constant weight at 70 C, ground to 60 mesh in a Wiley Mill, and stored in a desicator. Starch in the tissue was determined by the procedure of Neilsen (1943) and Neilsen and Gleason (1945) as modified by Young (H. Y. Young, unpublished methods, Pineapple Research Institute of Hawaii, 1949). A known weight of sample (50 to 100 mg) was taken depending on the amount of starch present in the tissue as estimated by the intensity of color development in a tissue subsample with potassium iodide indicator solution. Starch was extracted with 4.4 ml of 60% perchloric acid for 15 minutes, diluted to 40 ml with distilled water, and centrifuged at 1500 rpm for three minutes. A 5.0 ml aliquot of the supernatant was pipetted into a 250 ml conical flask, diluted with 15 ml of distilled water, and titrated with 2.0 N NaOH until just alkaline using phenolphthalein as the indicator. Four ml of 2.0 N acetic acid was added immediately after titration. After that, 0.2 ml of 50% potassium iodide and 5 ml of 0.01 N KIO_3 were added and solution was diluted to 50 or 100 ml with distilled water depending on the intensity of the blue color. After standing for five minutes, absorbance was determined at 590 nm.

Since different starches give starch iodine colors of varying quality and intensity, purified pineapple starch was used to prepare a standard curve which ranged from 5 to 40 ppm starch. The starch content of pineapple tissue was expressed on a percent dry weight basis.

Purified pineapple starch was obtained from H. Y. Young, former

chemist of the Pineapple Research Institute of Hawaii. The unpublished method for the preparation of purified pineapple starch is as follow. The mid-section of fresh pineapple stem was cut into 2 cm pieces and blended for one to two minutes with an adequate amount of water. Strain the homogenate through four layers of "Purity cheesecloth", grade 90. Let the suspension stand for 15 minutes to settle the starch. Decant most of the supernatant, add water, resuspend, decant again and repeat until the supernatant is colorless. Transfer the sample to a 250 ml centrifuge bottle with water and centrifuge at about 1000 rpm for three minutes. Decant, add 100 ml of 0.1 N NaOH and suspend the sample by stirring and shaking. Centrifuge, decant and repeat this alkali wash. Wash once with water and then twice with 0.1 N HCl in a similar maner. Wash the sample twice with water. Finally suspend the starch in 1:1 ether-alcohol, centrifuge, decant and repeat. Drain the residue for about 10 minutes, air dry and then place in a 70 C oven over night. Grind to a powder in a mortar and dry to constant weight at 105 C. A white powder is obtained which stains deep blue with iodine. Purity should be checked by microscopic examination and by HCl hydrolysis to glucose followed by the Quisumbing-Thomas method for reducing sugar (H. Y. Young, unpublished method, Pineapple Research Institute of Hawaii).

Determination of Total Soluble Sugars

Total soluble sugars in the middle one-third of the D-leaf and in the top and middle one-third of the stem were assayed according to

the method of Dubois et al. (1956) as modified by Haslemore and Roughan (1976). The plant tissue was oven dried at 70 C to constant weight, ground to 60 mesh and stored in a desiccator. About 100 mg of the plant sample was extracted with 10 ml of 62.5% methanol for 15 minutes at 55 C. The samples were cooled and centrifuged at 2000 rpm for three minutes. A 4.0 ml aliquot was removed and transferred to a second series of testubes. Five ml of chloroform was added and the tubes were capped and shaken vigorously. The samples were briefly centrifuged to aid phase separation. A 100 ul aliquot was removed from the upper aqueous phase and added to 2.0 ml of 5% phenol. Eight ml of concentrated sulphuric acid was added rapidly against the liquid surface to obtain good mixing. The samples were allowed to cool to room temperature. After mixing well, the absorbance was measured at 490 nm. Total soluble sugars calculated from a standard curve, were expressed as percent dry weight of the plant material.

The standard curve was prepared by diluting 0.5, 1.0 and 1.5 ml of a sucrose standard (10 mg/ml in 62.5% methanol) solution to 10 ml with 62.5% methanol. A 4 ml aliquot was removed and treated in the same manner as the unknowns to give standards equivalent to 5, 10, and 15% soluble sugars on a dry weight basis.

Determination of Fructose, Glucose and Sucrose

Fructose, glucose and sucrose in the leaf and stem tissues were analysed following the procedures of Brandao et al., (1980) and Richmond et al., (1981). A 250 mg dry sample was extracted with 15 ml of 80% ethanol for 30 minutes at 55 C. The samples were cooled

and centrifuged at 300 rpm for five minutes. A 6.0 ml aliquot was transferred to a second series of test tubes and evaporated to dryness. The samples were then made to 4.0 ml volume with 0.01 M $CaSO_4$ solution and filtered through a 0.45 μm Metrical membrane (Gelmann Filtration Products, Ann Arbor, MI) to ensure removal of any particulate impurities that might be present. After that, the samples were passed through Sep-Pak C_{18} cartridges (Waters Associates, Inc., Milford, Massachusetts) to remove the color pigments. The Sep-Pak cartridge was placed at the end of a 10 ml graduated syringe. The cartridge was first prewetted with 2.0 ml of acetonitrile and then flushed with 5.0 ml of distilled water, followed by two to three volumes of air before the sample was placed into the syringe. The first 2.0 ml of sample was discarded and the second 2.0 ml of sample was collected for high-performance liquid chromatographic analysis.

The analysis of sugars was carried out using two bonded phase carbohydrate columns and a differential refractometer detector (Waters Model RI-401). The mobile phase was 0.01 M $CaSO_4$. One ml samples were loaded onto the column with a Micromeritics 725 Auto Injector. The column temperature was maintained at 83 C. Detector attenuation was held constant at 4x and a Hewlett Packard Integrator (HP 3390 A) was used for recording the detector response.

Ethylene Measurements

A 2.0 ml gas samples was assayed for ethylene on a gas chromatograph equipped with an alumina column and a flame ionization

detector. Concentration of ethylene in the samples was obtained by comparing the peak height of the sample to a 100 ppm ethylene standard.

DATA ANALYSIS

Data were analysed by analysis of variance for Experiment I and by general linear model (GLM) (SAS User's guide, 1979) for Experiment II and III. Statistical analysis of percentage of forcing was done after arcsin transformation (degree). However, presentation and interpretation were based on actual percentage of forcing.

CHAPTER IV

RESULTS AND DISCUSSION

EXPERIMENT I

Effects of Nitrogen on Growth and Physiological Status of the Plants

Pineapple plants having different levels of leaf nitrogen were established to determine the effects of nitrogen on physiological status and on susceptibility of the plants to forcing with ethephon. Although levels of nitrogen in the D-leaves were significantly different, due to the short term the treatments were imposed, there was no significant effect of nitrogen on plant dry weight or moisture content (Table 4). Dry weight did increase somewhat with increasing nitrogen.

Leaf chlorophyll concentration measured on the day of forcing increased linearly and significantly with increasing leaf nitrogen (Table 5). Leaf chlorophyll concentration as a function of leaf nitrogen was described by the equation:

chlorophyll = 0.0781 + (0.1221) leaf nitrogen

and the R^2 value was 0.88. The results were similar to those of Tam and Magistad (1935) who obtained chlorophyll contents in pineapple leaves that ranged from 0.106 to 0.375 mg g^{-1} fresh weight as applied nitrogen increased. Maftoun et al. (1980) also obtained nearly maximum levels of leaf chlorophyll in three kinds of ornamental plant having Crassulacean acid metabolism when nitrogen was increased from 0 to 100 ppm. A further increase in nitrogen to 200 ppm slightly

TABLE 4. Effect of treatment on leaf nitrogen content, moisture content and dry weight of leaves and stem.

Leaf nitrogen[a]		Moisture content[b]		Dry weight[b]	
		Leaf	Stem	Leaf	Stem
		- - - - % - - - -		- - - - g - - - -	
	0.96 %	86.6	84.2	116.3	18.2
	1.39 %	87.1	80.2	121.3	21.3
	1.72 %	86.6	79.6	134.4	23.7
	2.05 %	85.8	80.8	135.6	22.2
S.E.[c]	0.032	0.376	1.389	5.774	1.638

[a] Each value is an average of nine observations.

[b] Each value is an average of six observations.

[c] Standard error of the mean.

TABLE 5. Effect of nitrogen on leaf chlorophyll and titratable acids of pineapple.

Leaf nitrogen	Chlorophyll[a] fresh wt. basis	Titratable acids[a]	
		7:00 a.m.	5:00 p.m.
	- mg g^{-1} -	- meq 100 g^{-1} fresh wt. -	
0.96 %	0.195	22.3	3.4
1.39 %	0.250	23.6	4.2
1.72 %	0.292	22.3	4.5
2.05 %	0.327	20.3	4.3
S.E.[b]	0.013	1.458	0.274

[a] Each value is an average of nine observations.

[b] Standard error of the mean.

increased the chlorophyll content in Kalanchoe laxiflora and Sedum telephoides but decreased the chlorophyll content in Kalanchoe verticillata.

Levels of leaf nitrogen had no significant effect on titratable acids at 7:00 a.m. or at 5:00 p.m. (Table 5). Acid loss during the day ranged from 81 to 83 percent.

The levels of leaf titratable acids and percent reduction of the acids during the day were comparable to values obtained by Sideris et al. (1948) for eight month old pineapple plants grown in solution cultures under greenhouse conditions. However, Sideris and Young (1947) obtained significantly higher levels of titratable acids from leaves of pineapple plants supplied with 140 ppm nitrogen than from plants supplied with 2.8 ppm nitrogen in the solution culture. No data were given by the authors on levels of nitrogen in leaves but they did note that the low nitrogen plants were sufficiently stressed for nitrogen that leaf die-back occurred (Sideris and Young, 1946). The differences in the results of this experiment and those of Sideris and Young (1947) were probably due to the much smaller differences between levels of nitrogen supplied to the plants in this study.

Total soluble sugars in leaves (dry weight basis) collected at 5:00 p.m. were highest in the treatment which had the lowest leaf nitrogen content (Table 6). Sugars decreased linearly and significantly to a minimum of 8.5% as the level of leaf nitrogen increased to a maximum. The relationship between leaf nitrogen and

TABLE 6. Effect of nitrogen on total soluble sugars of leaf and stem of pineapple.

Leaf nitrogen	Total soluble sugars		
	Leaf[a]	Stem (top)[b]	Stem (middle)[b]
	- - - - - - - % dry weight - - - - - - -		
0.96 %	15.0	13.1	10.8
1.39 %	12.0	13.0	10.9
1.72 %	10.2	12.8	10.3
2.05 %	8.5	12.9	8.7
S.E.[c]	0.937	0.704	0.134

[a] Each value is an average of nine observations.

[b] Each value is an average of six observations.

[c] Standard error of the mean.

soluble sugars was described by the equation:

leaf total soluble sugars = 20.3129 + (-5.7973) leaf nitrogen

and the R^2 value was 0.75.

Sideris et al. (1948) found that total soluble sugars in leaves reached maximum and minimum values at 6:00 p.m. and and 6:00 a.m. respectively. Under field conditions leaf total soluble sugars of nine month old pineapple plants at 3:00 p.m. ranged from 18.5 to 28.6% (Sideris et al., 1948), levels much higher than those found in this experiment. In an earlier study with plants grown in solution culture, Sideris and Young (1947) reported values of total soluble sugars in the leaf comparable to those shown in Table 6. They obtained 16.0% total soluble sugars in the leaves of pineapple plants grown in solution culture supplied with 140 ppm nitrogen. Leaf sugars decreased to 8.3% in plants supplied with 2.8 ppm nitrogen. The differences in results obtained by previous workers and those reported here could be partly due to differences in growing conditions, plant size, and stage of plant development.

Total soluble sugars in the top of the stem decreased slightly as the level of leaf nitrogen increased but the differences were not significant. Total soluble sugars in the middle third of the stem decreased linearly and significantly with increasing leaf nitrogen. Total soluble sugars in the middle part of the stem as a function of leaf nitrogen was described by the equation:

total soluble sugars = 12.9874 + (-1.8470) leaf nitrogen

and the R^2 value was 0.61. The slightly lower total soluble sugars

in the middle than in the top third of the stem were in agreement with the results of Sideris et al. (1939).

The effect of nitrogen nutrition on the starch content of leaves and stem (Table 7) was similar to that on total soluble sugars. Leaf starch decreased linearly and significantly with increasing leaf nitrogen. Leaf starch as a function of leaf nitrogen was described by the equation:

leaf starch = 3.0120 + (-0.6977) leaf nitrogen

and the R^2 value was 0.71. The results suggest that in the plants having high nitrogen, carbohydrates were being used for growth rather than being converted to storage forms. Starch in the top and middle thirds of the stem were also highest at the lowest nitrogen level and it decreased as the level of nitrogen increased but the differences were not significant. The tendency towards higher levels of starch and total soluble sugars in plants having low nitrogen indicates there was less utilization of carbohydrates in these plants than in the high nitrogen plants, a result consistent with the results of others (Sideris et al., 1948).

Ethylene Evolution

Ethylene evolution from pineapple having different levels of nitrogen was measured to investigate the effect of nitrogen on rate of ethylene production from the plants and to assess its relationship, if any, to flowering. Ethylene evolution at night from the pineapple plants before application of ethephon was 0.120, 0.255,

TABLE 7. Effect of nitrogen on starch content of leaf and stem of pineapple.

Leaf nitrogen	Starch		
	Leaf[a]	Stem (top)[b]	Stem (middle)[b]
	- - - - - - - % dry weight - - - - - - -		
0.96 %	2.3	13.6	16.9
1.39 %	2.1	13.4	14.1
1.72 %	1.8	11.3	13.5
2.05 %	1.6	10.0	13.4
S.E.[c]	0.107	2.086	1.854

[a] Each value is an average of nine observations.

[b] Each value is an average of six observations.

[c] Standard error of the mean.

0.191, and 0.273 μl plant^{-1} hr^{-1} in plants having 0.96, 1.39, 1.72 and 2.05% nitrogen, respectively, in the D-leaves. Ethylene evolution tended to increase as the levels of nitrogen increased but the differences were not significant (P = 0.05) and the trend was not consistent.

Plant tissues which normally evolve little or no ethylene may produce large amounts of ethylene as a result of stress such as cutting (Meigh et al., 1960; Saltveit and Dilley, 1978), bruising (Hanson and Kende, 1976), contact with some chemicals (Yu and Yang, 1980; Kimmerer and Kozlowski, 1982), and other stresses (Abeles, 1973; Lieberman, 1979). Burg and Burg (1966) were unable to detect ethylene evolution from pineapple (cv. Pernambuco) before application of naphthaleneacetic acid. Different cultivars may also produce different amounts of ethylene. In this experiment, the plants were put into smaller chambers than those used by Burg and Burg (1966) (30 liters compared to 40 liters) and some leaves were trimmed to facilitate sealing the plants into the chambers. Ethylene evolution from plants before application of ethephon could have been due to a wound response.

After application of 0.5 mg of ethephon, ethylene production increased slightly to about 0.5 μl plant^{-1} hr^{-1}, but increased sharply with the application of 5.0 mg ethephon per plant (Figure 1). At the high rate of ethephon, significantly more ethylene was released from high than from low nitrogen plants. The level of ethylene decreased progressively with time and reached near minimum

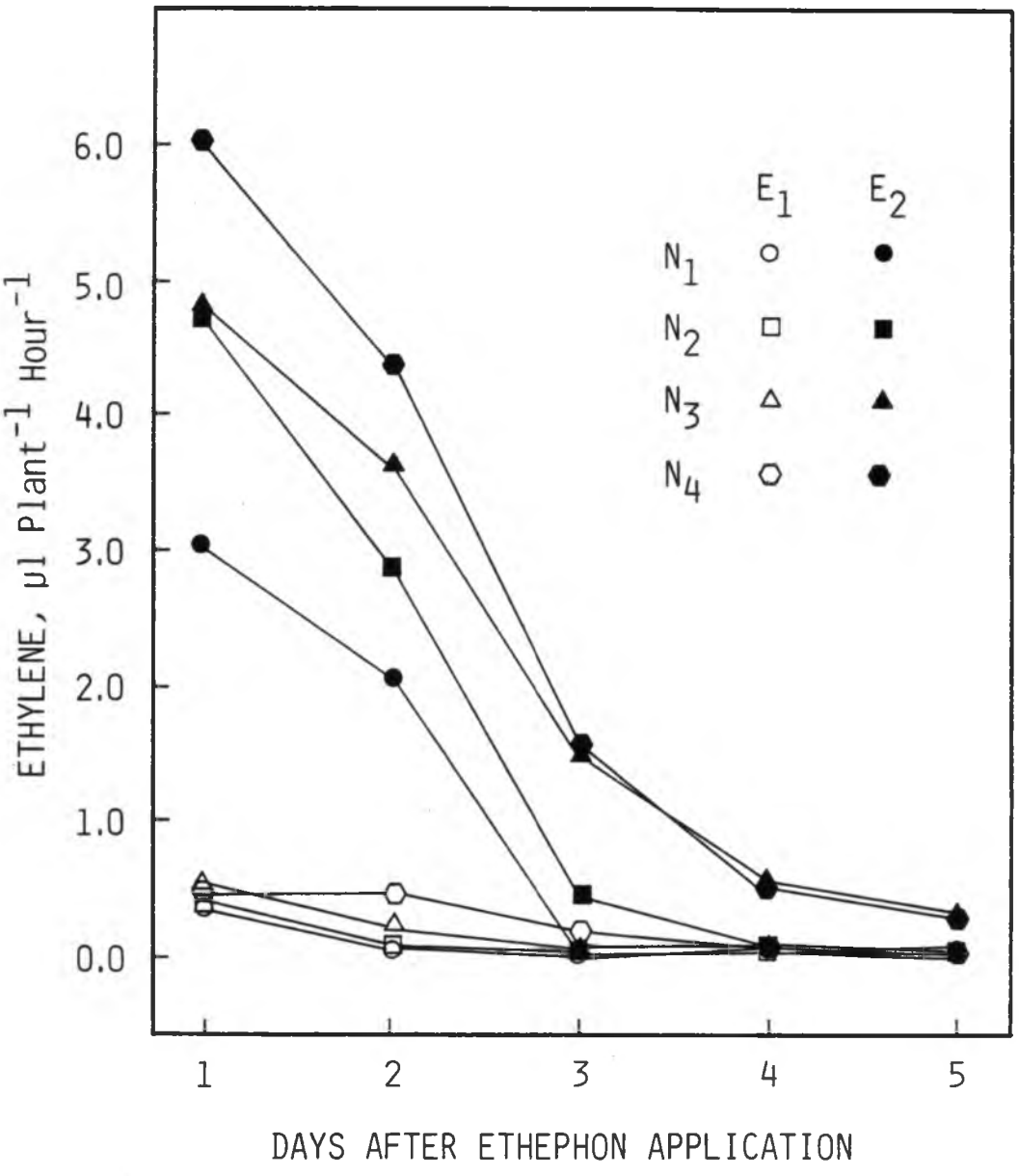

FIGURE 1. Effect of nitrogen and ethephon on ethylene evolution from pineapple plants after application of 0.5 (E_1) or 5.0 mg (E_2) ethephon. Levels of leaf nitrogen were 0.96, 1.39, 1.72 and 2.05% for treatments N_1, N_2, N_3 and N_4 respectively. Each value is an average of three observations.

levels three to four days after treatment. The trend was similar for all levels of nitrogen. The level of ethylene evolution from plants treated with 0.5 mg ethephon and the lack of any apparent response to leaf nitrogen resulted in a significant interaction ($P = 0.01$) between the level of leaf nitrogen and ethylene evolution.

Similar patterns of ethylene evolution from other plant tissues treated with ethephon were obtained by several workers (Young and Jahn, 1975; Epstein et al., 1977; Yamaguchi et al., 1971; Ben-Tal and Lavee, 1976). High initial levels of ethylene evolution were mostly from decomposition of applied ethephon after which evolution declined to a minimum within three to five days after treatment under outdoor conditions (Yamaguchi et al., 1971; Ben-Tal and Lavee, 1976) and in 14 to 20 days in laboratory experiments (Young and Jahn, 1975; Epstein et al., 1977). In contrast, when the majority of the evolved ethylene was induced internally by the application of ethephon, ethylene evolution was high for more than five days while ^{14}C-ethylene evolution from ^{14}C-ethephon rapidly declined to a low level within five days after treatment (Yamaguchi et al., 1971). Assuming that ethylene production from pineapple plants was more or less continuous and declined approximately linearly over time, the total amount of ethylene evolution from a plant having the highest level of nitrogen during the five day period would be 0.315 ml. The total volume of ethylene that would be produced by complete degradation of 5.0 mg of ethephon would be 0.846 ml (at a temperature of 25 C and pressure of 1 atmosphere). The higher rate of ethylene evolution

from plants receiving greater amounts of nitrogen could be partly due to the effect of residual urea fertilizer on the pH of the solution in the center of the plant. Urea and ammonia produced from urea by hydrolysis could increase the pH of the applied ethephon solution and thus increase the rate of ethephon decomposition. The results suggest that the high rate of ethylene evolution from pineapple plants during first two to three days after application of ethephon was primarily from break down of the applied ethephon. The inability to account for more than about 50% of the applied ethephon could be due to incomplete sealing at the base of the plant chambers or to other unknown causes.

Response to Ethephon

The percentage of plants forced with ethephon, averaged over levels of nitrogen, decreased significantly as ethephon decreased (Table 8). Percent forcing commonly increases when rates of ethephon are increased (Guyot and Py, 1970; Py and Guyot, 1970; Dass et al., 1975; Dass et al., 1976; Ouattara, 1982). However, the effective rate may depend on the method of application, environmental conditions in each location, cultivar, and physiological status of the plants.

Percent forcing, averaged over levels of ethephon, increased as leaf nitrogen increased from 0.96 to 1.39% and then decreased as nitrogen was increased further. However, only high nitrogen treatment had a significantly ($P = 0.05$) lower forcing percentage

TABLE 8. Effects of nitrogen and ethephon on inflorescence initiation (forcing) and development of pineapple

Treatment	Forcing	Inflorescence[a] length	Peduncle diameter	Florets per inflorescence
	- % -	- - - - mm - - - -		
Ethephon				
0.5 mg	71.5	37.0	13.3	89.6
5.0 mg	84.7	41.6	13.6	92.8
S.E.[b]	4.804	1.751	0.345	0.307
Leaf nitrogen				
0.96 %	77.8	43.5	14.4	91.2
1.39 %	92.9	40.8	14.2	94.4
1.72 %	81.0	38.6	13.8	89.6
2.05 %	60.7	34.3	11.4	89.6
S.E.	5.548	2.022	0.398	0.355

[a] Total length of the peduncle and developing inflorescence.

[b] Standard error of the mean.

than the other treatments. The nitrogen by ethephon interaction was not significant. The optimum level of leaf nitrogen for flower induction with ethephon appears to be around 1.4%. Susceptibility of pineapple plants to ethephon forcing decreased significantly when leaf nitrogen increased to 2.1%. However, the decrease in percentage of flowering in high nitrogen plants was not as striking as the reduction observed by Aldrich and Nakasone (1975) who obtained a 52% decrease in flowering percentage from high nitrogen plants compared to low nitrogen plants. However, Aldrich and Nakasone (1975) did not include the level of leaf nitrogen in their report. The difference in the results could be partly due to differences in growing conditions. The plants in this experiment were grown in pots and kept in the greenhouse while Aldrich and Nakasone (1975) reported on studies conducted in the field. Although the plants in the pots absorbed large amounts of nitrogen, they may not have been able to utilize the nitrogen efficiently for growth. This fact is suggested by the relatively high starch level in the plants in this experiment compared to those plants of similar dry weight of Conway (1977).

Increased amounts of ethephon significantly ($P = 0.05$) increased inflorescence length but not diameter (Table 8). Other workers have shown that the rate of inflorescence development increases with increasing rates of ethephon (Py and Guyot, 1970; Ouattara, 1982).

Inflorescence length decreased significantly as the levels of nitrogen increased from 0.96 to 2.05%. Inflorescence length as a function of leaf nitrogen was described by the equation:

inflorescence length = 50.7435 + (-7.4604) leaf nitrogen

and the R^2 value was 0.27, indicating that only a small fraction of the variation in inflorescence length was attributable to the effect of nitrogen. There were similar trends for the effect of nitrogen on peduncle diameter. However, only the high nitrogen treatment had a significantly smaller peduncle diameter than the others. Peduncle diameter decreased more or less linearly as leaf nitrogen increased and the relationship was described by the equation:

peduncle diameter = 17.5471 + (-2.6477) leaf nitrogen

and a rather low R^2 value of 0.43 was obtained. The nitrogen by ethephon interaction was not significant (P = 0.05) for either inflorescence length or peduncle diameter. Increasing levels of nitrogen have been reported by others to retard the rate of inflorescence development (Py and Guyot, 1970; Bondad, 1973).

It is not possible to determine whether the slow rate of inflorescence development for the high nitrogen plants was due to slower initiation of the inflorescence after application of ethephon or to a slower rate of the inflorescence growth after floral initiation took place. However, higher levels of starch and sugars in the low nitrogen plants may have contributed to the higher susceptibility of the pineapple to ethephon forcing and the more rapid growth of the inflorescence.

Neither levels of nitrogen nor rates of ethephon or their interaction had any significant effect on the number of florets per inflorescence. The lack of an effect of either treatement on floret

number suggests that low ethephon and high nitrogen may retard rate of development, but so long as levels of ethphon or nitrogen are adequate, they do not appear likely to affect final fruit size.

EXPERIMENT II

This study was conducted to examine the effect of night temperature and duration of exposure on the physiological characteristics of the pineapple plant and on forcing success with ethephon. There were no significant effects of night temperature on dry weights or moisture contents of leaves and stems of pineapple although plants exposed to a 30 C night temperature had slightly lower dry weights than plants exposed to 20 C (Table 9). Conway (1977) also found no significant effect of short-term exposure to night temperature on fresh and dry weight of pineapple plants. However, where plants were grown continuously at different night temperatures, Friend (1981) reported that plants grown at 30 C were significantly smaller than those grown at 20 and 25 C.

Stem moisture content, averaged over night temperatures, decreased slightly as duration of exposure to night temperature increased (Table 9). Plants exposed to the night temperature treatments for three weeks had a significantly lower average stem moisture content than plants exposed for only one week. Leaf and stem dry weight of the plants exposed to the night temperature treatments for three weeks were also significantly higher than for plants exposed to one or two weeks. However, there were no

Table 9. Effects of night temperature and duration of exposure to night temperature on moisture content, and dry weight of leaves and stem of pineapple.

Main treatment effects	Moisture content		Dry weight	
	Leaf	Stem	Leaf	Stem
	- - - - % - - - -		- - - - g - - - -	
Night Temperature				
20 C	86.2[a]	86.3	122.5	12.0
25 C	86.6	86.5	122.6	11.7
30 C	86.2	85.8	114.1	11.7
Duration of Exposure				
1 Wk	86.5[b]	86.6	114.9	11.4
2 Wk	86.3	86.3	113.5	10.6
3 Wk	86.1	85.7	130.9	13.4
Experiment S.E.	0.223	0.233	5.130	0.573

[a] Each mean is an average of nine samples averaged over three durations of exposure.

[b] Each mean is an average of nine samples averaged over three night temperatures.

significant effects of the duration of exposure to night temperature on leaf moisture content. The temperature by duration of exposure interaction for leaf and stem dry weight and moisture content also were not significant.

The significantly lower average stem moisture content of the plants exposed to the night temperature treatments for three weeks could be due to the fact that plants exposed for three weeks were larger than plants for the other durations. Stem moisture content was linearly related to stem dry weight and was described by the equation:

stem moisture content = 90.6202 + (-0.3745) stem dry weight

and the R^2 value of 0.64 was highly significant (P = 0.0001).

Leaf nitrogen contents of the chlorophyllous middle one-third of the D-leaf on the day of forcing increased linearly and significantly as the night temperature increased (Table 10) though the correlation was relatively low (R = 0.57). Leaf nitrogen as a function of night temperature was described by the equation:

leaf nitrogen = 1.1200 + (0.0193) night temperature

with an R^2 value of 0.33. Leaf nitrogen also tended to increase as the duration of exposure to night temperature increased but the differences were not significant at the 5% level. The temperature by duration of exposure interaction also was not significant.

Conway (1977) found no significant effect of night temperature on total leaf nitrogen content. The differences in the results obtained here and those of Conway (1977) might be partly due to

Table 10. Effects of night temperature and duration of exposure to night temperature on leaf nitrogen and titratable acids of pineapple.

Main treatment effects	Total N[a]	Titratable acids[b]	
		7:00 a.m.	5:00 p.m.
	- % -	- meq 100 g^{-1} fresh wt. -	
Night Temperature			
20 C	1.52	36.7	5.8
25 C	1.59	31.3	6.5
30 C	1.71	18.2	4.4
Duration of Exposure			
1 Wk	1.57	28.2	5.8
2 Wk	1.60	30.1	5.6
3 Wk	1.64	27.9	5.3
Experiment S.E.	0.036	0.833	0.239

[a] Each value is an average of 36 samples.

[b] Each value is an average of 27 samples.

differences in the nutrient solution used to grow the pineapple plants and to the greater number of the samples collected in this study.

Leaf titratable acids measured at 7:00 a.m., averaged over duration of exposure to night temperature, decreased linearly as nignt temperature increased (Table 10) and the relationship was described by the equation:

titratable acids = 75.1185 + (-1.8556) night temperature

and R^2 value (R^2 = 0.87) was highly significant. Titratable acids approximately doubled as temperature increased from 20 C to 30 C. Duration of exposure to night temperature had no significant effect on titratable acids. The temperature by duration of exposure interaction for titratable acids measured at 7:00 a.m. also was not significant.

Although statistical analysis showed that night temperature significantly affected the level of titratable acids at 5.00 p.m., the trends were not consistent. The highest level of titratable acids at 5:00 p.m. was obtained from plants exposed to a 25 C night temperature. Plant held at 30 C had significantly lower levels of titratable acids. The temperature by duration of exposure interaction was not significant.

The results support the reports of others (Bennet-Clark, 1933; Thomas and Ranson, 1954; Seshagiri and Suryanarayanamurthy,1957; Ranson and Thomas, 1960; Neales, 1973, Conway, 1977) that titratable acids are significantly higher and the intensity of Crassulacean acid

metabolism (CAM) is greater when dark period temperatures are low.

Leaf starch was highest in plants exposed to a 20 C night temperature and decreased more or less linearly as temperature increased (Table 11). Leaf starch as a function of night temperature was described by the equation:

leaf starch = 6.4815 + (-0.1482) night temperature

and the correlation was significant (P = 0.01). The R^2 value was 0.53 indicating that just slightly more than 50% of the variation in starch was accounted for by the effects of night temperature. Effects of duration of exposure to night temperature and temperature by duration interaction were not significant.

A similar relationship between levels of starch, averaged over duration of exposure, and night temperature was found for starch in the middle part of the stem (Table 11). Starch in the middle part of the stem as a function of night temperature was described by the equation:

starch = 36.2954 + (-0.9550) night temperature

and the correlation was highly significant (P = 0.01). The R^2 value was 0.57. There were no significant effects of duration of exposure to night temperature on levels of starch in the middle parts of the stem. The temperature by duration interaction also was not significant.

In the top part of the stem, main effects could not be evaluated unambiguously because the temperature by duration of exposure interaction was significant. The significant temperature by duration

Table 11. Effects of night temperature and duration of exposure to night temperature on starch content in leaf and stem of pineapple.

Main treatment effects	Starch	
	Leaf[a]	Stem (middle)[b]
	- - - - - % dry weight - - - - -	
Night Temperature		
20 C	3.4	16.7
25 C	3.0	13.4
30 C	1.9	7.2
Duration of Exposure		
1 Wk	2.7	13.6
2 Wk	3.0	11.4
3 Wk	2.6	12.3
Experiment S.E.	0.183	0.823

[a] Each value is an average of 27 samples.

[b] Each value is an average of nine samples.

of exposure interaction resulted from the fact that starch increased with duration of exposure in plants exposed to 20 C and decreased with duration of exposure in plants held at 30 C (Table 12).

Total soluble sugars in D-leaves, averaged over duration of exposure to night temperature, also decreased significantly with increasing night temperature (Table 13). Values for plants exposed to 25 and 30 C decreased 5.3 and 25%, respectively, relative to values measured at 20 C. Plants exposed to the 30 C night temperature had significantly ($P = 0.01$) lower total soluble sugars than plants from the other treatments.

Total soluble sugars in the top part of the stem, averaged over duration of exposure to night temperature, decreased with increasing night temperature (Table 13). Plants held at a 30 C night temperature had significantly lower total soluble sugars in the top part of the stem than plants at 20 C. The middle part of the stem had slightly lower total soluble sugars than the top part but changes associated with night temperature were similar (Table 13). The plants held at a 20 C night temperature had significantly ($P = 0.05$) higher total soluble sugars in the middle part of the stem than plants from the other two treatments.

No significant effect of duration of exposure to night temperature was observed on the level of total soluble sugars in the leaf or stem and the temperature by duration interaction also was not significant.

Table 12. Effects of night temperature and duration of exposure to night temperature on starch content in the top one-third of the stem of pineapple.

Night temperature	Duration of exposure (week)		
	1	2	3
	- - - - - - % Dry Weight - - - - - -		
20 C	10.8[a]	12.6	15.8
25 C	11.2	12.6	11.5
30 C	11.0	8.2	8.1
S.E.[b]	0.975		

[a] Each value is an average of three observations.

[b] Standard error of the mean.

Table 13. Effects of night temperature and duration of exposure to night temperature on total soluble sugars content in leaf and stem of pineapple.

Main treatment effects	Total soluble sugars		
	Leaf[a]	Stem (top)[b]	Stem (middle)[b]
	- - - - - - % dry weight - - - - - -		
Night Temperature			
20 C	13.2	17.5	14.5
25 C	12.5	16.6	12.7
30 C	9.9	15.6	12.5
Duration of Exposure			
1 Wk	11.9	16.8	13.8
2 Wk	11.2	16.6	12.7
3 Wk	12.4	16.3	13.1
Experiment S.E.	0.590	0.428	0.349

[a] Each value is an average of 27 samples.

[b] Each value is an average of nine samples.

Ethylene Evolution

Rates of ethylene evolution at night from plants held at night temperatures of 20, 25 and 30 C before application of ethephon were 0.217, 0.168, and 0.187 μl $plant^{-1}$ hr^{-1} respectively and the levels were similar to those measured previously in Experiment I. No significant treatment effects were detected.

After application of ethephon, ethylene evolution increased greatly on the first day, and the rate increased significantly with increasing night temperature (Figure 2). Ethylene evolution from plants in all treatments decreased progressively with time.

On the third day after treatment with ethephon, plants exposed to the 30 C night temperature evolved significantly ($P = 0.05$) less ethylene than plants at 20 C. It is likely that ethephon remaining in the center of plants held at 30 C decomposed more rapidly during the first two days than ethephon applied to plants held at the other temperatures. Thus, on the third day, less ethephon would be available for decomposition to ethylene in the plants held at 30 C. Ethylene evolution from the plants continued to decrease, and by the fourth day, had reached rates similar to those measured prior to ethephon application.

There was no significant effect of duration of exposure to night temperature on ethylene evolution from the plants. The temperature by duration of exposure interaction also was not significant.

Lougheed and Franklin (1970) showed that ethylene evolution from tomato fruits treated with ethephon at the "breaker stage" but held

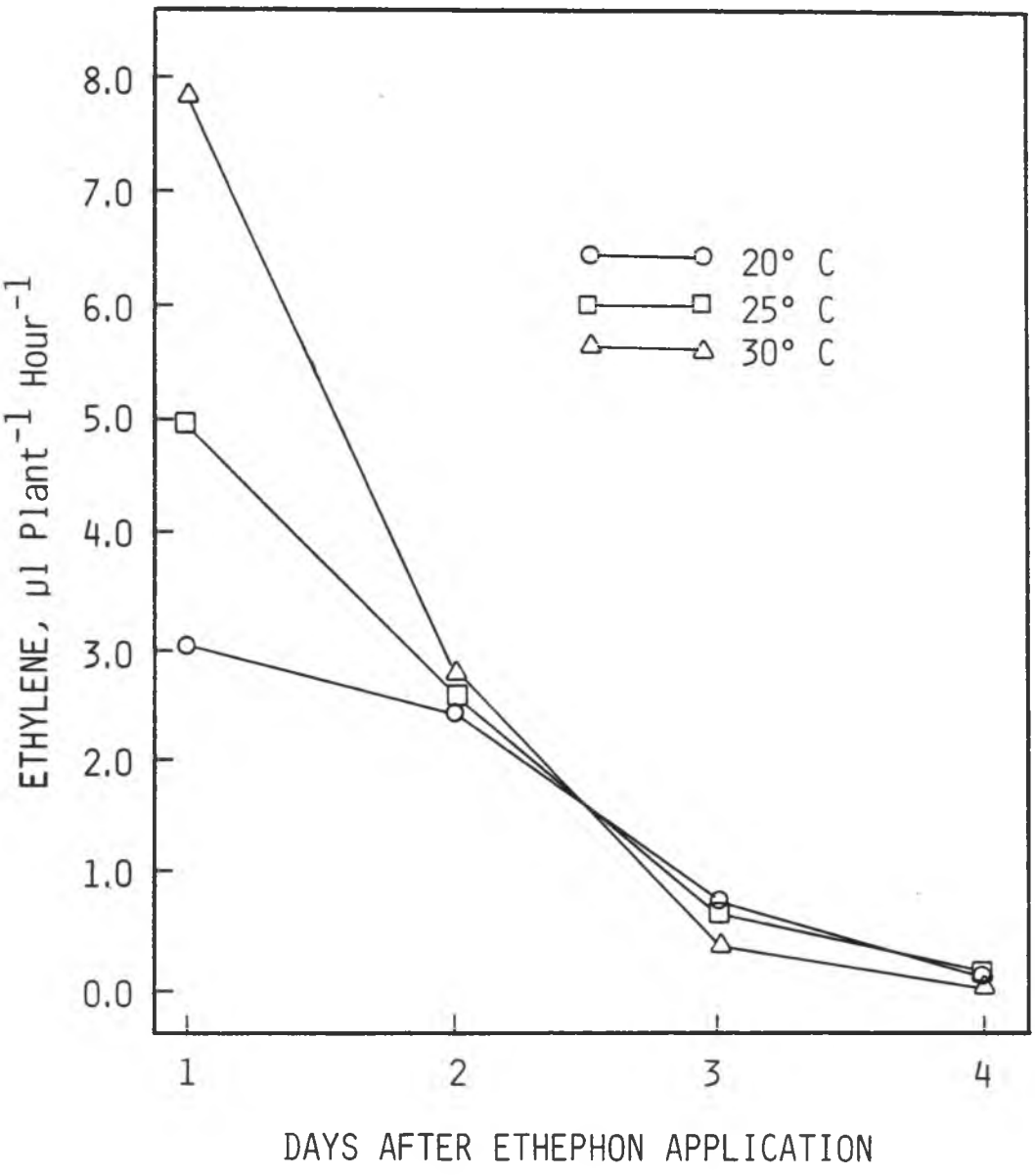

Figure 2. Effect of night temperature, averaged over duration of exposure, on ethylene evolution from pineapple plants after application of 5.0 mg of ethephon. Each point is the mean of nine observations.

under a nitrogen atmosphere was equal to the difference in ethylene production of ethephon treated fruits held in air and untreated fruits held in the air. They concluded that breakdown of ethephon in plant tissue is a chemical process and the amount of ethylene from the breakdown of ethephon was a function of the amount of ethephon absorbed rather than the weight of the plant tissue. In some cases, ethephon can stimulate internal production of ethylene from plant tissue. In tomato plants, ethylene evolution was high for more than five days while ^{14}C-ethylene evolution from ^{14}C-ethephon rapidly declined to a minimum within five days after treatment (Yamaguchi et al., 1971).

The rate of ethephon breakdown into ethylene both in vitro and in vivo is temperature dependent and increases with increasing temperature from 15 to 55 C (Biddle et al., 1976; Klein et al., 1978; Olien and Bukovac, 1978).

The pattern of ethylene evolution from pineapple plant after application of ethephon was similar to the results of Yamaguchi et. al. (1971) for summer squash and Young and Jahn (1975) for citrus fruit. Ethylene production from summer squash and citrus fruit was highest during the first or second day after ethephon application and ethylene production rapidly declined to a low level after four to five days. The data for pineapple plants in Experiment II were also more or less similar to those of Experiment I which suggests that a high rate of ethylene production from pineapple during first two to three days was principally due to break down of the applied ethephon.

Effect of Night Temperature on Forcing

The effect of night temperature on percent forcing was determined by dissecting the plants at 39 days after forcing. Forcing was expressed as a percentage and the data were analyzed statistically after arcsine transformation; however, the interpretation of the results was based on actual forcing percentages.

Forcing percentage was reduced 25% by a night temperature of 30 C and the effect was significant (Table 14). Temperatures of 25 and 20 C had no significant effect on percent forcing. Also no significant effects of duration of exposure to night temperature or the temperature by duration of exposure interaction were detected.

The results were more or less similar to the results of Conway (1977) who obtained a reduction in forcing percentage at 30 C from six month old plants but the differences in forcing among plants at night temperatures of 20, 25 and 30 C were relatively small. However, Conway (1977) also obtained a trend of lower forcing for plants exposed to a 25 C night temperature than for plants exposed to 20 C. The differences in the results of this experiment and those of Conways (1977) could be due to the differences of nutrient solution applied to the plants. Moving of the plants in and out of the temperature controlled chambers in Conway's (1977) experiment may also have contributed to the differences of the results.

In Queensland, when the daily maximum temperature was higher than 30 C, ethephon in a high pH buffer solution was ineffective in inducing inflorescence initiation in pineapple. However, the low pH

Table 14. Effects of night temperature and duration of exposure to night temperature on inflorescence initiation (forcing), inflorescence length, peduncle diameter, and number of florets per inflorescence in pineapple.

Main treatment effects	Forcing	Inflorescence[a] length	Peduncle diameter	Florets per inflorescence
	- % -	- - - - - mm - - - - -		
Night Temperature				
20 C	97.2	63.2	16.9	84.6
25 C	100.0	70.7	17.8	84.2
30 C	75.0	68.1	16.0	76.6
Duration of Exposure				
1 Wk	83.3	71.1	17.1	82.4
2 Wk	94.4	67.0	17.0	81.8
3 Wk	94.4	64.0	16.6	81.2
Experiment S.E.	4.835	4.339	0.459	1.358

[a] Total length of the peduncle and developing inflorescence.

treatment (24 mg ethephon plus urea solution), which slowed the rate of ethephon decomposition, still gave nearly maximum percent flower induction (Glennie, 1980). High temperature which caused the rapid breakdown of ethephon into ethylene (Biddle et. al., 1976) also decreased the effectiveness of ethephon in reducing fruit removal force in olive (Klein et. al., 1978).

The lower percent forcing of plants at the 30 C night temperature could have been due to the rapid break down of ethephon into ethylene and loss of ethylene from the plants. However, the lower levels of soluble sugars and starch may also play a part in reducing the susceptibility of the plants to forcing at the 30 C night temperature. The somewhat lower forcing percentage for plants exposed to the night temperature treatments for one week than for two of more weeks suggests that plants which have less time to adapt to the new environments in the temperature controlled chambers may have reduced susceptibility to forcing with ethephon.

There was no significant effect of night temperature or duration of exposure on inflorescence length. However, inflorescence length averaged over duration of exposure tended to increase with increasing night temperature from 20 to 25 C and then decreased as night temperature increased further (Table 14).

Night temperatures of 20 and 25 C had no significant effect on peduncle diameter but diameter was significantly smaller at a night temperature of 30 C (Table 14). Peduncle diameter averaged over level of night temperature tended to decrease with increasing

duration of exposure to night temperature but the effect was not significant.

Number of florets per inflorescence decreased with increasing night temperature and plants held at 30 C had a significantly lower number of florets per inflorescence (Table 14).

Plants held at high night temperature had reduced CO_2 assimilation during the night as indicated by levels of titratable acid in the morning. High night temperature would also increase the rate of respiration, thus reducing carbohydrate reserves in the plant. A lower number of florets per inflorescence in plants held at a 30 C night temperature could be the result of low carbohydrate reserves in the plants.

EXPERIMENT III.

Since both night temperature and nitrogen influence plant susceptability to forcing, Experiment III was conducted to study the interaction between nitrogen and night temperature.

Physiological Status of the Plant

The effects of night temperature on leaf and stem moisture content and stem dry weight were not significant. However, leaf dry weight of plants exposed to the 20 C night temperature was significantly ($p = 0.05$) greater than the average leaf weight for plants from the other treatments (Table 15). The greater leaf weight for the 20 C treatment was more likely related to differences in

Table 15. Effects of treatment on leaf nitrogen content, moisture content, and dry weight of leaves and stem of pineapple.

Main treatment effects	Total N[a]	Moisture content[b]		Dry weight[b]	
		Leaf	Stem	Leaf	Stem
	- - - - - - % - - - - - -			- - - g - - -	
Night Temperature					
20 C	1.85	86.3	82.9	136.5	17.3
Ambient[c]	1.82	86.3	83.5	118.1	15.0
30 C	1.88	86.0	84.4	115.3	15.4
S.E.	0.024	0.266	0.607	5.632	0.960
Leaf nitrogen					
N_1	1.06	86.3	83.3	118.3	16.3
N_2	2.64	86.1	83.9	128.3	15.5
S.E.	0.020	0.219	0.501	4.645	0.792

[a] Each value is an average of 30 samples for night temperature means and 45 samples for nitrogen means.

[b] Each value is an average of six samples for night temperature means and nine samples for nitrogen means.

[c] The average temperature for this treatment was 22.5 C.

plant size prior to establishing the treatments than to treatment effects.

The effects of night temperature on leaf nitrogen also were not significant (Table 15). This results are in contrast to those of Experiment II where leaf nitrogen was significantly higher for plants expose to 30 C night temperature than for plants expose to 20 and 25 C. There are no obvious explanations for the differences in results between the two experiments.

Level of nitrogen in the D-leaves due to the treatments were significantly different but as was found for Experiment I, the effects of nitrogen on leaf and stem moisture contents and dry weights were not significant (Table 15). Differences in weight and moisture content were not expected since the treatments were of relatively short duration. However, leaf dry weight tended to increase with increasing levels of nitrogen.

Leaf chlorophyll concentration was significantly higher for plants held at a night temperature of 30 C than for plants held at the two lower temperatures (Table 16). The effect of night temperature on leaf chlorophyll concentration was not studied in Experiment II but leaf nitrogen increased with increasing night temperature in that experiment.

Leaf chlorophyll concentration increased significantly with increasing nitrogen (Table 16). The results were similar to those of Experiment I and those of other workers (Tam and Magistad, 1935; Maftoun et al., 1980). No significant temperature by nitrogen

Table 16. Effects of night temperature and nitrogen on leaf chlorophyll and titratable acids of pineapple.

Main treatment effects	Chlorophyll[a]	Titratable acids[a]	
		7:00 a.m.	5:00 p.m.
	- mg g^{-1} -	- meq 100 g^{-1} fresh wt. -	
Night temperature			
20 C	0.348	32.2	4.5
Ambient[b]	0.333	31.8	4.4
30 C	0.418	23.0	4.6
S.E	0.011	1.036	0.226
Leaf nitrogen			
1.06 %	0.279	27.6	3.7
2.64 %	0.454	30.4	5.3
S.E.	0.009	0.855	0.186

[a] Each value is an average of 24 samples for night temperature means and 36 samples for nitrogen means.

[b] The average temperature for this treatment was 22.5 C.

interaction for leaf chlorophyll was detected

Leaf titratable acids at 7:00 a.m. were significantly lower for plants exposed to a night temperature of 30 C than for plants exposed to ambient temperature and to 20 C (Table 16). Levels of leaf titratable acids at 5:00 p.m. were not affected by night temperature. The results were similar to those of Experiment II and those of Conway (1977).

Leaf titratable acids increased with increasing nitrogen both at 7:00 a.m. and 5:00 p.m. The results are in agreement with those of Sideris and Young (1947) but different from results obtained in Experiment I where levels of titratable acids at 7:00 a.m. were not affected by nitrogen. The differences could be partly due to the fact that effect of nitrogen on titratable acids in this experiment was averaged over three levels of night temperature while in Experiment I, all plants were maintained at the ambient greenhouse temperature. However, the temperature by nitrogen interaction for titratable acids both at 7:00 a.m. and 5:00 p.m. was not significant.

Leaf starch was approximately linearly related to night temperature (Table 17) and the relationship was described by the equation:

leaf starch = 7.2168 + (-0.1874) night temperature

and the R^2 value was 0.48. The results were comparable to and consistent with those of Experiment II where leaf starch content decreased with increasing night temperature.

High nitrogen plants also had significantly ($p = 0.05$) lower

Table 17. Effects of night temperature and nitrogen on starch content in pineapple leaf and stem tissue.

Main treatment effects	Starch	
	Leaf[a]	Stem (top)[b]
	- - - - % dry weight - - - -	
Night temperature		
20 C	3.5	10.3
Ambient[c]	2.9	9.1
30 C	1.6	7.5
S.E	0.253	0.987
Leaf nitrogen		
1.06 %	3.0	11.6
2.64 %	2.3	6.3
S.E.	0.209	0.814

[a] Each value is an average of 30 samples for night temperature means and 45 samples for nitrogen means.

[b] Each value is an average of six samples for night temperature means and nine samples for nitrogen means.

[c] The average temperature for this treatment was 22.5 C.

leaf starch than plants with low leaf nitrogen (Table 17). The effect of leaf nitrogen on leaf starch content was in agreement with the results of Experiment I but leaf starch values in this experiment were somewhat higher than those of Experiment I. The different nutrient solution and or a longer photoperiod or both could possibly account for the relatively higher starch levels in leaves in this experiment. There was no significant temperature by nitrogen interaction for leaf starch.

Starch in the top one-third of the stem decreased with increasing night temperature but the differences were not significant (Table 17). Although the trend was similar to data for Experiment II, the values in this experiment were slightly lower than those obtained in the earlier experiment. Plants exposed to 30 C night temperature in Experiment II also had significantly lower starch in the top part of the stem than plants exposed to other temperatures. More variation of starch content in the top of the stem in this experiment (C.V. = 26.5%) than was obtained from plants in Experiment II (C.V. = 15.0%) may have contributed to the nonsignificant different in starch in the top of the stem in this experiment.

High nitrogen plants had significaltly ($p = 0.01$) lower starch in top part of the stem than did the low nitrogen plants (Table 17). The values in this experiment were also slightly lower than those obtained from experiment I. However, in Experiment I, the effect of leaf nitrogen on starch content in the top part of the stem was not significant. The difference in the results of this experiment and

those obtained for Experiment I could be partly due to the fact that the effect of nitrogen on starch in the top of the stem was averaged over night temperatures in this experiment. The temperature by nitrogen interaction was not significant.

There was a significant (P = 0.01) temperature by nitrogen interaction on starch content in the middle part of the stem. Low nitrogen plants had similar starch contents in the middle part of the stem at all levels of night temperature, but in high nitrogen plants starch decreased significantly with increasing night temperature (Table 18). Starch values in the middle part of the stem averaged over night temperature are somewhat higher than those obtained for Experiment I. The reason for a significant temperature by nitrogen interaction effect on starch in the middle of the stem and the absence of such an interaction in the top part of the stem is not clear. The coefficient of variation for starch in the middle of the stem was only 8.4% while it was 26.5% for the top one-third. The reason for the large amount of variation in starch levels in the top of the stem also is not known. The care was taken to use fresh solution and to check the accuracy of the method with standards, part of the variation may have been due to inaccuracies in the starch analysis.

No clear relationship was found between susceptibility to forcing of plants with ethephon and total soluble sugars or starch in the D-leaf or in stem tissue in Experiments I and II. Therefore, in Experiment III, analyses of sucrose, glucose and fructose in leaf and

Table 18. The effects of night temperature and nitrogen on starch content in the middle one-third of the stem of pineapple.

Treatment	Night temperature		
	20 C	Ambient[a]	30 C
Leaf nitrogen			
1.06 %	19.4[b]	20.3	20.1
2.64 %	18.1	15.1	10.4
S.E.[c]	0.834		

[a] The average temperature for this treatment was 22.5 C.

[b] Each value is an average of 15 samples.

[c] Standare error of the mean.

stem tissue were run to study the relationship between susceptibility to forcing and levels of sugars in leaf and stem.

Leaf fructose decreased significantly as night temperature increased (Table 19). Plants exposed to a 20 C night temperature had significantly lower fructose than plants from the other treatments. High nitrogen plants also had significantly lower leaf fructose than low nitrogen plants (Table 19). The temperature by nitrogen interaction was not significant.

There were significant temperature by nitrogen interactions for leaf total soluble sugars, sucrose and glucose (Table 20). Leaf total soluble sugars decreased at greater degree in high nitrogen plants than in low nitrogen plants as night temperature increased from 20 to 30 C. A significant temperature by nitrogen interaction for leaf sucrose was obtained because leaf sucrose was higher in high nitrogen plants than in low nitrogen plants maintained at 20 C. Leaf sucrose for both high and low nitrogen plants decreased to similar values at 30 C.

Leaf glucose levels were similar (absent) in high nitrogen plants at all levels of night temperature while the values for low nitrogen plants decreased significantly with increasing night temperature. This differential response at the two nitrogen levels accounted for the significant temperature by nitrogen interaction for leaf glucose.

Although levels of leaf total soluble sugars decreased with both increasing night temperature and nitrogen, the responses of each

Table 19. Effects of night temperature and nitrogen on fructose content in pineapple leaves.

Main treatment effects	Fructose[a]
	- % dry weight -
Night temperature	
20 C	6.87
Ambient[b]	6.27
30 C	3.95
S.E	0.596
Leaf nitrogen	
1.06 %	9.31
2.64 %	2.08
S.E.	0.492

[a] Each value is an average of 30 samples for night temperature means and 45 samples for nitrogen means.

[b] The average temperature for this treatment was 22.5 C.

Table 20. Effects of night temperature and nitrogen on total soluble sugars, sucrose and glucose in pineapple leaves.

Treatment	Night temperature		
	20 C	Ambient[a]	30 C
Leaf nitrogen	Leaf total soluble sugar (% dry wt.)[b]		
1.06 %	16.5	15.9	11.7
2.64 %	5.8	5.1	3.1
S.E.[c]	0.306		
	Leaf sucrose (% dry wt.)[b]		
1.06 %	2.6	1.5	1.3
2.64 %	3.2	2.7	1.4
S.E.	0.157		
	Leaf glucose (% dry wt.)[b]		
1.06 %	7.1	5.3	3.2
2.64 %	(.)[d]	(.)	(.)
S.E.	0.320		

[a] The average temperature for this treatment was 22.5 C.

[b] Each value is an average of 15 samples.

[c] Standard error of the mean.

[d] None detected.

individual sugar to increasing night temperature and nitrogen were different. Leaf sucrose, glucose (except in high nitrogen plants) and fructose decreased by approximately the same percentages (29 to 55 %) when night temperature increased from 20 to 30 C. In contrast, leaf sucrose increased while glucose and fructose decreased, the latter by up to 78% when leaf nitrogen increased from 1.06 to 2.64%. The data suggest that high respiration rates or low CAM activity during the night or both occurred at the high night temperature since increasing night temperature affected all sugars in more or less the same proportion. The results of the effects of nitrogen on leaf sugars suggest that nitrogen metabolism was carried on at the expense of leaf glucose, and to a lesser degree, fructose while leaf sucrose was increased.

Total soluble sugars were higher in the top than in the middle third of the stem (Table 21 and 22). Soluble sugars were significantly higher in both parts of the stem in plants exposed to a 20 C night temperature than in plants held at 30 C. Low nitrogen plants also had significantly more total soluble sugars in both parts of the stem than plants having high nitrogen. The temperature by nitrogen interactions were not significant for either part of the stem.

In the top part of the stem, sucrose was highest in plants exposed to the 20 C night temperature (Table 21). Sucrose decreased with increasing night temperature and plants exposed to a 30 C night temperature had significantly lower sucrose than plants at 20 C.

Table 21. Effects of night temperature and nitrogen on sucrose, glucose, and total soluble sugars content in the top one-third of the stem of pineapple.

Main treatment effects	Sucrose[a]	Glucose[a]	Total soluble sugars[a]
	- - - - - - % dry weight - - - - - -		
Night temperature			
20 C	8.90	2.50	18.5
Ambient[b]	7.37	1.58	16.1
30 C	6.75	2.25	15.7
S.E	0.513	0.176	0.497
Leaf nitrogen			
1.06 %	8.30	3.31	19.5
2.64 %	7.04	0.91	14.0
S.E.	0.423	0.145	0.410

[a] Each value is an average of 30 samples for night temperature means and 45 samples for nitrogen means.

[b] The average temperature for this treatment was 22.5 C.

Table 22. Effects of night temperature and nitrogen on glucose and total soluble sugars content in the middle one-third of the stem of pineapple.

Main treatment effects	Glucose[a]	Total soluble sugars[a]
	- - - % dry weight - - -	
Night temperature		
20 C	2.77	12.2
Ambient[b]	2.35	10.5
30 C	2.17	10.7
S.E	0.218	0.443
Leaf nitrogen		
1.06 %	3.24	12.3
2.64 %	1.61	10.1
S.E.	0.179	0.365

[a] Each value is an average of 30 samples for night temperature means and 45 samples for nitrogen means.

[b] The average temperature for this treatment was 22.5 C.

Glucose also decreased with increasing night temperatures but plants maintained in ambient temperature had a significantly lower level of glucose than plants at 20 C (Table 21). There was no obvious explanation for the higher glucose level in the top part of the stem for plants at 30 C than for plants at ambient temperature.

Levels of sucrose and glucose in the top part of the stem were lowest in plants having high nitrogen, but the effects were significant ($p = 0.01$) only for glucose (Table 21).

The effect of night temperature on glucose in the middle one-third of the stem was not significant although levels tended to decrease with increasing night temperature (Table 22). High nitrogen plants had significantly ($P = 0.01$) lower glucose in the middle one-third of the stem than plants having low nitrogen.

There was a significant temperature by nitrogen interaction for fructose in the top one-third of the stem (Table 23). Fructose levels were more or less similar in low nitrogen plants as night temperature increased from 20 to 30 C, but decreased significantly in high nitrogen plants as night temperature increased from 20 to 30 C.

Significant temperature by nitrogen interactions were also obtained for sucrose and fructose in the middle one-third of the stem. Sucrose in the middle one-third of the stem of low nitrogen plants increased with increasing night temperature while the values in high nitrogen plants decreased with increasing night temperature (Table 23).

For fructose in the middle one-third of the stem, the values

Table 23. Effects of night temperature and nitrogen on fructose in the top one-third, sucrose and fructose in the middle one-third of the stem of pineapple.

Treatment	Night temperature		
	20 C	Ambient[a]	30 C
	- - - - - - % dry weight - - - - - -		
Leaf nitrogen	Stem (top) fructose[b]		
1.06 %	3.6	2.4	3.6
2.64 %	1.4	0.5	(.)[c]
S.E.[d]	0.311		
	Stem (middle) sucrose[b]		
1.06 %	3.5	3.7	4.1
2.64 %	4.4	3.4	3.3
S.E.	0.272		
	Stem (middle) fructose[b]		
1.06 %	2.5	2.3	2.7
2.64 %	2.2	2.1	1.1
S.E.	0.224		

[a] The average temperature for this treatment was 22.5 C.

[b] Each value is an average of three samples.

[c] None detected.

[d] Standard error of the mean.

were similar for low nitrogen plants at all night temperatures but decreased with increasing night temperature in high nitrogen plants (Table 23).

Ethylene Evolution

Before application of ethephon, ethylene evolution from the pineapple plants, averaged over levels of nitrogen was 0.119 µl $plant^{-1}$ hr^{-1} from plants exposed to the 30 C night temperature and decreased to 0.075 and 0.050 µl $plant^{-1}$ hr^{-1} for plants at 20 C and ambient temperature, respectively. Ethylene evolution averaged over night temperatures was 0.083 and 0.077 µl $plant^{-1}$ hr^{-1} from plants with high and low nitrogen, respectively but the differences were not significant. Ethylene evolution from the plants in this experiment was lower than evolution from plants in Experiments I and II. Reduced ethylene evolution could have been due to a reduction in wound ethylene produced as a result of trimming the leaves before putting the plants into the chambers. In Experiments I and II the leaves were trimmed on the day of measurement while in this experiment, trimming of the leaves was done three days before measurement.

After application of ethepon, ethylene evolution increased greatly and on the first day was significantly ($p = 0.01$) greater from plants exposed to the 30 C night temperature and decreased with decreasing night temperatures (Figure 3). Ethylene evolution decreased more of less progressively over time reaching minimum

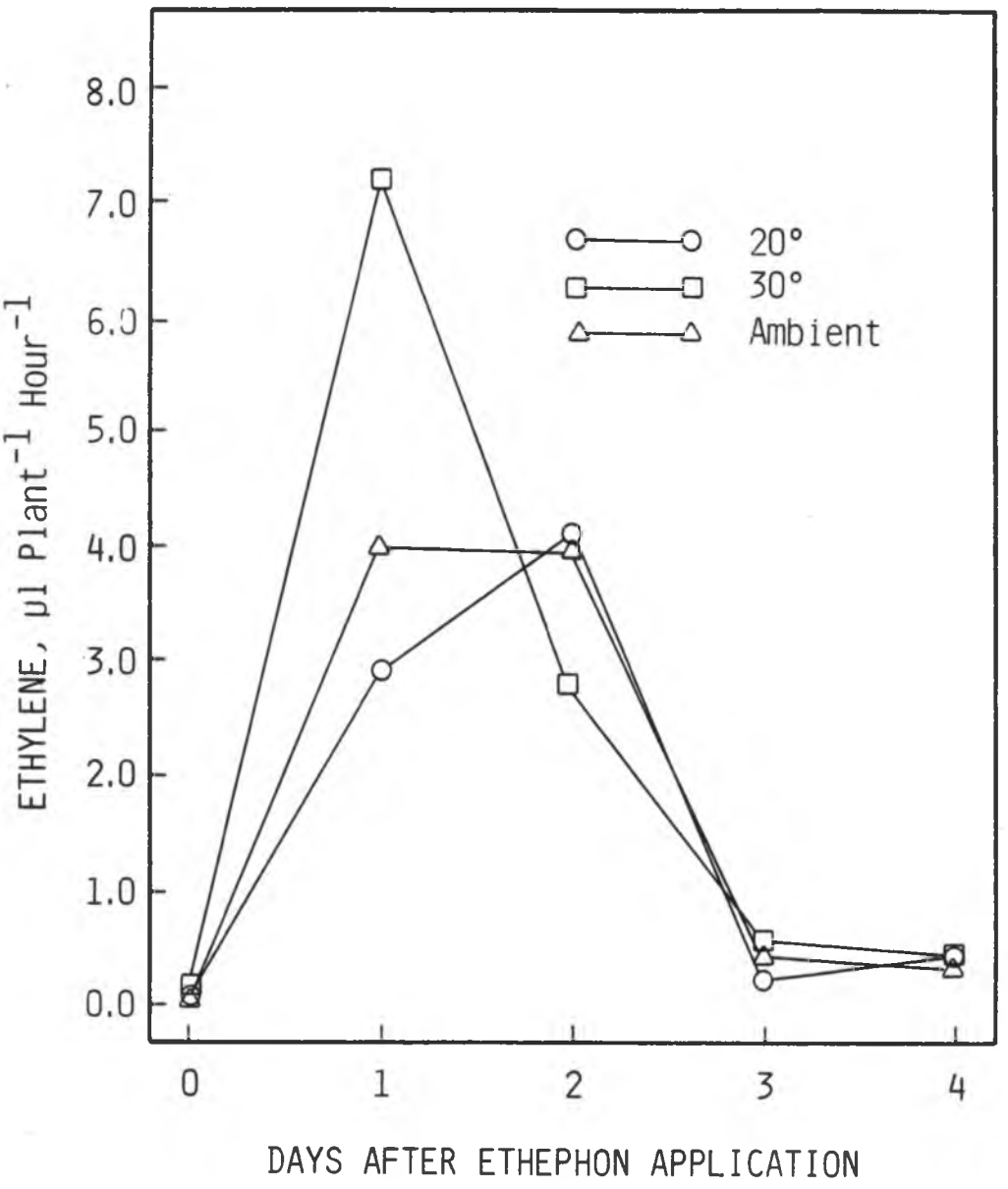

Figure 3. Effect of night temperature, averaged over two levels of nitrogen, on ethylene evolution from pineapple plants after application of 5.0 mg ethephon. Each point is an average of six samples.

levels by three days after treatment. There is no obvious reason for the increase in ethylene evolution between the first and second day for plants at 20 C.

Ethylene evolution from plants having high and low nitrogen averaged over night temperature also decreased progressively with time and reached minimum levels three days after treatment. Plants having a high level of nitrogen evolved more ethylene than those with the low level and the differences were significant ($p = 0.01$) on the second and fourth days after ethephon application (Figure 4). The temperature by nitrogen interaction were significant for all four days after ethephon application but the trend were not consistent.

The results were more or less consistent with those of the previous experiments both with respect to the pattern and the rate of ethylene evolution; i.e., a high initial rate mostly from decomposition of the applied ethephon followed by a progressive decrease over time reaching low levels at about three to four days after treatment.

Flowering Response

The forcing percentage, averaged over levels of nitrogen, was highest for plants maintained at a 20 C night temperature (Table 24). Percentage forcing decreased with increasing night temperature but the results were not significantly different. The effect of nitrogen and the interaction between night temperature and nitrogen on the percentage of plants forced also were not significant. Plants having

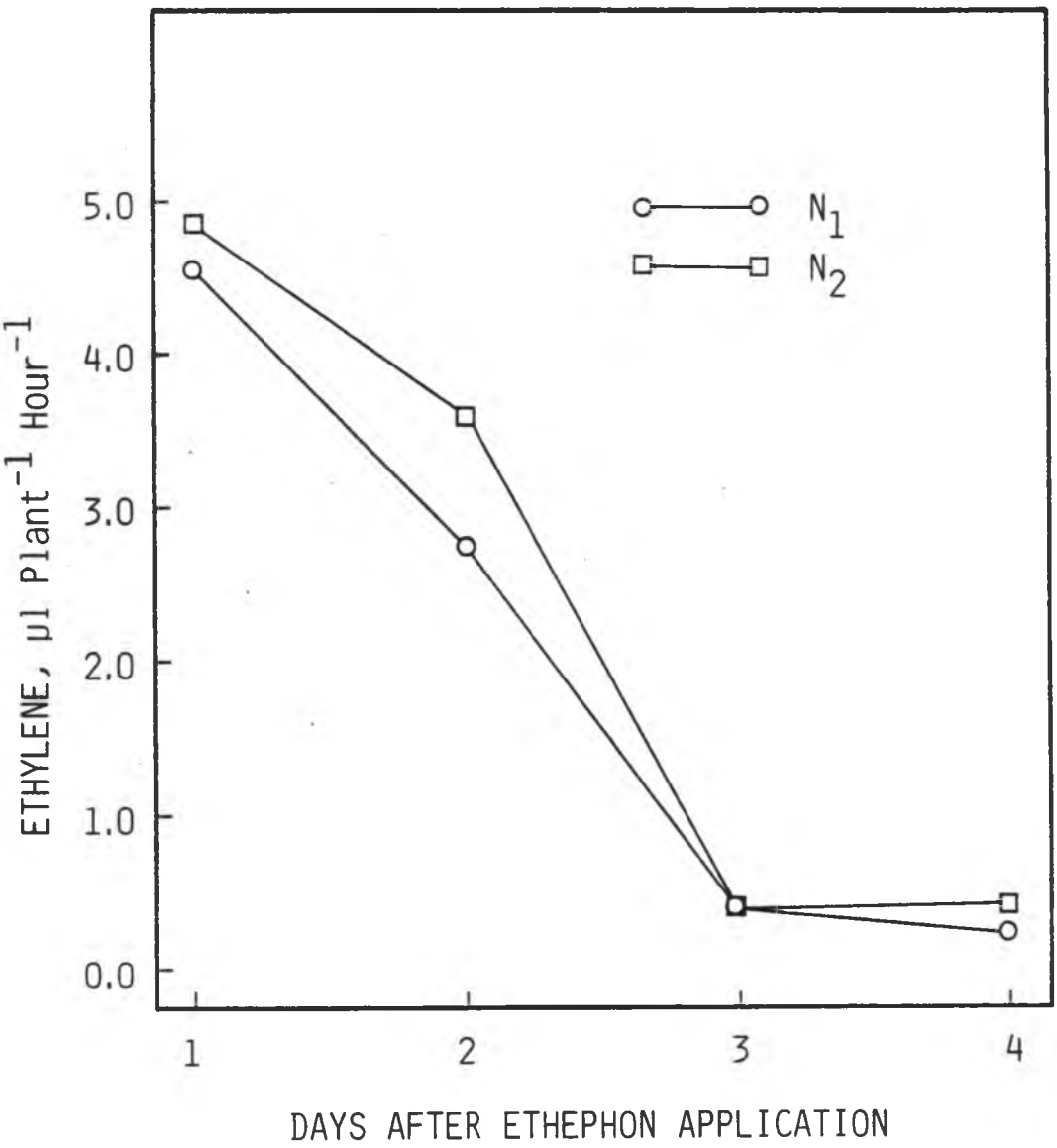

Figure 4. Effect of nitrogen, averaged over three levels of night temperature, on ethylene evolution from pineapple plants after application of 5.0 mg ethephon. Levels of leaf nitrogen were 1.06 and 2.64% for treatments N_1 and N_2, respectively. Each point is an average of nine samples.

Table 24. Effects of night temperature and nitrogen on inflorescence initiation (forcing), inflorescence length, peduncle diameter, and number of florets per inflorescence in pineapple.

Main treatment effects	Forcing	Inflorescence[a] length	Peduncle diameter	Florets per inflorescence
	- % -	- - - - mm - - - -		
Night temperature				
20 C	96.7	57.1	16.1	82.3
Ambient[b]	86.7	54.3	15.5	80.7
30 C	83.4	54.9	14.1	68.8
S.E	4.052	2.739	0.330	1.502
Leaf nitrogen				
1.06 %	88.9	57.5	15.5	76.1
2.64 %	88.9	53.4	14.9	78.4
S.E.	3.342	2.259	0.272	1.238

[a] Total length of the peduncle and developing inflorescence.

[b] The average temperature for this treatment was 22.5 C.

low and high nitrogen in the leaves had similar forcing percentages. The unusually high forcing percentage for plants with high nitrogen in this experiment could be partly due to a nutrient or some other unknown stress during the experiment which may have increased the susceptibility of the plants to the forcing treatment. Because the forcing response was so high and uniform, it was not possible to establish a relationship between soluble or insoluble carbohydrates and susceptibility to the forcing agent.

Inflorescence lengths measured 36 days after forcing were not significantly affected by treatment. However, inflorescence length tended to be greaterer for plants in the 20 C night temperature and from in the low nitrogen treatment (Table 24).

The trend of longer inflorescences from plants with low nitrogen is in agreement with the results of Experiment I suggesting that high carbohydrate reserves favor rapid growth of the inflorescence. In contrast, the effects of night temperature were not consistent, i.e. inflorescence growth tended to increase with increasing night temperatures in Experiment II but tended to decrease with increasing night temperatures in Experiment III. The results suggest that high night temperature may at times delay time of inflorescence initiation and at other times increase the rate of inflorescence development.

Peduncle diameter and number of florets per inflorescence, averaged over levels of nitrogen, were significantly greater at a night temperature of 20 C than at 30 C. Both peduncle diameter and number of florets per inflorescence decreased with increasing night

temperature. The results are in agreement with those of Experiment II that high night temperature decreased the peduncle diameter and number of florets per inflorescence.

Nitrogen did not have any significant effect on peduncle diameter and number of florets per inflorescence. However, peduncle diameter tended to decrease and number of florets per inflorescence tended to increase as nitrogen was increased (Table 24).

In Experiment I, the effects of nitrogen on peduncle diameter and number of florets per inflorescence also were not significant and peduncle diameter also tended to be smaller in the high nitrogen plants than in the low nitrogen plants. However, number of florets per inflorescence in Experiment I tended to increase as leaf nitrogen increased from 0.96 to 1.39% but tended to decrease as leaf nitrogen increased further. The small differences in the trends in number of florets per inflorescence in Experiment I and Experiment III could possibly be due to the longer photoperiod prevailing during inflorescence development in Experiment III (13 hr) than in Experiment I (12 hr).

In general, as long as leaf starch was equal to or greater than about 1.6%, forcing success did not appear to be consistently associated with leaf or stem starch or total soluble sugars. However, high carbohydrate reserves were associated with rapid growth of the inflorescence in Experiment I and III and also tended to increase number of florets per inflorescence in all experiments.

The lack of any relationship between forcing and leaf glucose,

fructose or sucrose suggests that susceptibility to forcing was sufficiently high to mask any relationship between carbohydrate contents and forcing.

Based on the results presented in figures 1, 2, 3, and 4, a moderate rate of ethylene evolution (3 to 5 μl plant^{-1} hr^{-1}) from the plants during the first two days after application of ethephon would be most likely to result in a high forcing percentage of pineapple plants. Too high a rate of ethylene evolution may cause too rapid loss of the gas from the plant system, thus reducing the effectiveness of the applied ethephon. Too low a rate of ethylene evolution may also result in too low a level of ethylene available to the plants which also reduced the response of the pineapple plants to ethephon forcing.

High levels of carbohydrate reserves relative to the size of the plants, as compared to the four month old plants of Conway (1977), may result in a relatively high percentage of forcing for plants with high nitrogen or exposed to a 30 C night temperature. Analysis of individual plant leaves for nitrogen, starch and soluble sugars (especially leaf sucrose) at the time of forcing and the degree of forcing success for plants grown with a minimum of stress over a fairly wide range of temperature and nitrogen levels may provide more relevant data on relationship between flowering and the physiological status of the pineapple plants. Stem tissue analyses are precluded because of the need to sacrifice the plant in order to collect the sample.

APPENDIX A

EFFECT OF LEAF POSITION ON FLOWER INDUCTION OF PINEAPPLE WITH ETHYLENE AND ETHEPHON

Abstract

Ethephon at the rate of 5 mg applied as a small drop to the D-leaf or to the center of the pineapple plant was as effective a forcing treatment as the control treatment which consisted of applying 5 mg ethephon in 20 ml of 4% urea solution to the center of the plant. However, rate of inflorescence emergence was slower at 56 and 63 days after forcing than the control treatment. Ethylene equivalent to 5 mg ethephon (0.8 ml) on the D-leaf overnight was not effective in inducing floral initiation in pineapple.

Introduction

It is well known that ethylene and ethephon can be used to induce inflorescence initiation in pineaple plants. Ethephon tended to be more effective when sprayed over the whole plant with high volume (92 ml per plant) of 4% urea-water solution (Ouattara, 1982), or applied directly to the center of the plant with lower volume (50 ml per plant) of water solution (Dass et al., 1976). No data are available on what tissues on the plant absorb ethylene or ethephon and transmit the stimulus for the flower initiation process. This experiment was conducted to determined whether the equivalent amount of ethylene or ethephon applied to a D-leaf of the plant was as

effective a forcing treatment as pouring a solution into the center of the plant.

Materials and Methods

A randomized complete block experiment was installed on the Del Monte Kunia plantation on the island of Oahu to determine tissue susceptibility to ethephon or ethylene and to determine whether or not the physiological stimulus is translocated from leaf to growing point. The treatments consisted of A) injection of 0.8 ml of ethylene into a plastic bag sealed to a D-leaf over night, B) 5 mg ethephon in 20 µl of 4% urea solution applied as a spot on a D-leaf, C) 5 mg ethephon in 20 µl of 4% urea solution applied as a spot to the center of the plant, and D) 5 mg ethephon in 20 ml of 4% urea solution poured into the center of the plant (the control treatment). The treatments were replicated four times. There were eight plants per plot. The plant fresh weight at the time of forcing was estimated to range from 1.75 to 2.25 kg.

The treatments were applied to the plants at 6:00 p.m. on March 21, 1983. In treatment A, a D-leaf was enclosed in a plastic bag and sealed with sealing tape. The ethylene was injected into the bag with a graduated syringe fitted with 25 gauge needle. The bag was removed at 8:00 a.m. on the following morning.

Inflorescence counts were started 49 days after treatment. Weekly counts of the number of inflorescences continued until 91 days after forcing.

Results and Discussion

Forcing success was determined by counting the number of plants developing an inflorescence. Counts were made at the 'half inch open heart' stage where the inflorescence is just visible and the heart leaves have opened about one centimeter. The cumulative percentage of flowering increased with time for all ethephon treatments (Figure 5). Inflorescence initiation was most rapid for the control treatment and percentage forcing plateaued at 63 days after treatment. Percentage forcing of the other ethephon treatments plateaued at 70 days after treatment. However, there were no differences in cumulative percentage of forcing for the ethephon treatments 77 days after forcing. No forcing was obtained for the ethylene treatment at the time the experiment was terminated.

The ineffectiveness of the ethylene treatment most likely was due to loss of the gas from the bag sealed to the leaf. The presence of ridges on the leaf makes a tight seal difficult to obtain. In contrast, a small drop of ethephon solution applied to the center of the plant was almost as effective as the control, indicating that the center of the pineapple plant may be the more effective site for forcing. The effectiveness of the small drop of ethephon on a D-leaf or in a plant center may be attributed to the fact that ethephon is readily absorbed by plant tissue. The release of ethylene from the absorbed ethephon can continue for longer than 24 hours (Experiment I). The results demonstrate that ethephon or a stimulus induced by it moves to the growing point where inflorescence development occurs.

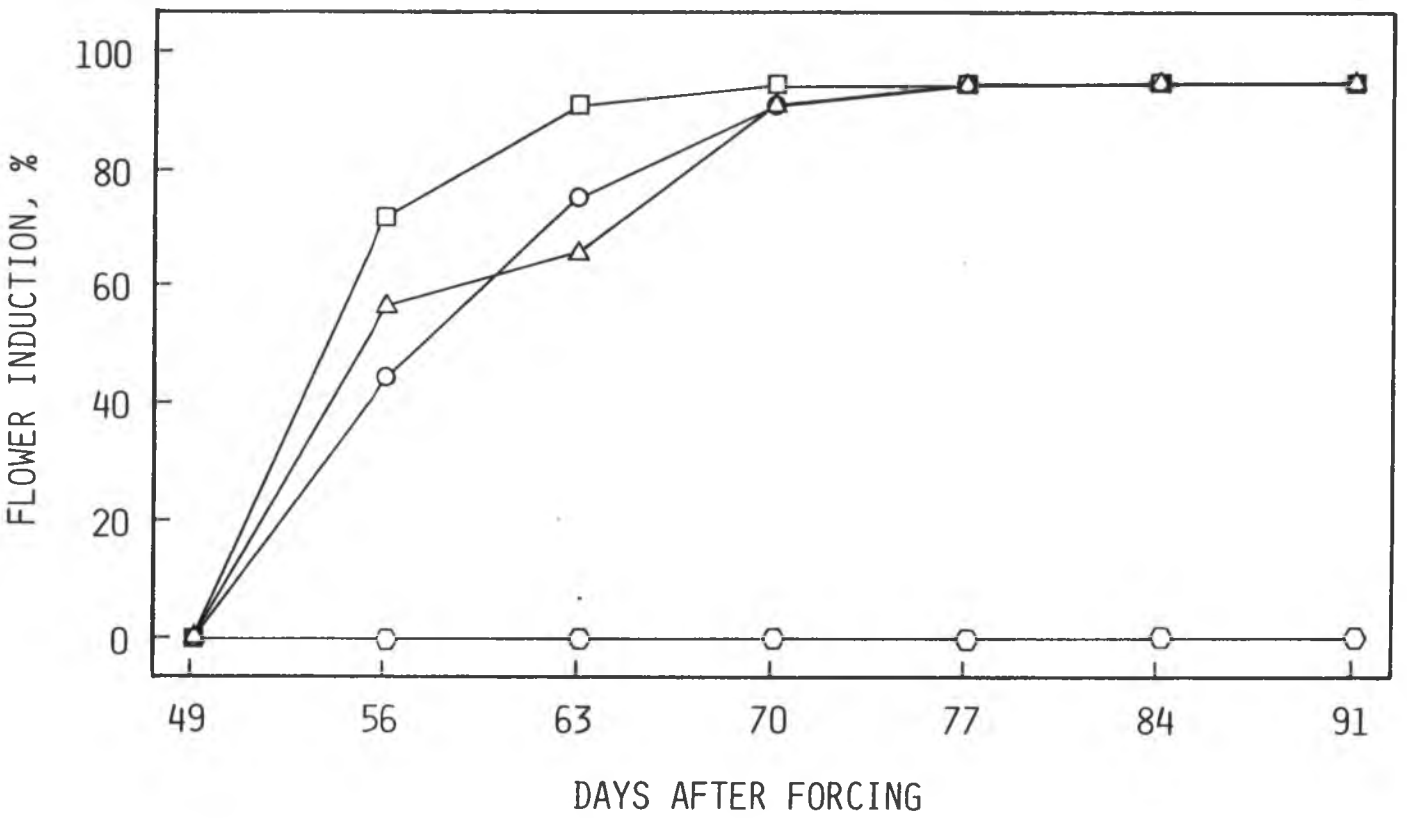

Figure 5. Cumulative percentage of inflorescence initiation of 'Smooth Cayenne' pineapple after treatment with 0.8 ml ethylene on a D-leaf over night (○), 5 mg ethephon in 20 µl of 4% urea solution applied to the center of the plant (○), 5 mg ethephon in 20 µl of 4% urea solution applied to the D-leaf (△), and 5 mg ethephon in 20 ml of 4% urea solution applied to the center of the plant as the control treatment (□). Data are the means for four replications.

APPENDIX B

Table 25. Effect of treatments on leaf nitrogen (**LFN**), leaf moisture content (**LFMC**), stem moisture content (**SMMC**), leaf dry weight (**LFDW**), stem dry weight (**SMDW**), leaf chlorophyll (**LFCH**), titratable acids at 7:00 a.m. (**TAMN**), and titratable acids at 5:00 p.m. Data are shown for three replications (**REP**).

TREATMENT	REP	LFN	LFMC	SMMC	LFDW	STDW	LFCH	TAMN	TAEN
		- - - - % - - - -			- - g - -		mg g^{-1}	meq 100 g^{-1}	
N_1	1	0.97	85.8	87.1	107.9	11.3	0.211	22.6	2.8
	2	0.96	87.2	86.8	107.9	18.8	0.180	20.3	3.2
	3	0.94	86.8	78.6	133.0	24.5	0.183	24.1	4.1
N_2	1	1.41	86.5	81.7	112.3	19.3	0.280	26.2	4.4
	2	1.41	87.8	79.2	122.1	22.7	0.254	19.4	3.8
	3	1.37	86.8	79.8	129.5	21.8	0.217	25.3	4.3
N_3	1	1.75	86.8	82.9	104.7	16.3	0.275	26.2	5.0
	2	1.76	86.3	77.2	146.7	27.0	0.312	18.9	4.1
	3	1.66	86.5	79.5	151.7	27.7	0.289	21.8	4.3
N_4	1	2.09	86.5	84.4	122.9	13.9	0.319	20.7	4.4
	2	2.16	85.7	80.7	140.1	25.1	0.326	21.9	4.4
	3	1.90	85.2	78.5	143.8	27.6	0.335	18.5	4.1

Table 26. Effect of treatments on leaf total soluble sugars (**LFTS**), stem (top) total soluble sugars (**TPTS**), stem (middle) total soluble sugars (**MDTS**), leaf starch (**LFST**), stem (top) starch (**TPST**), stem (middle) starch (**MDST**), and ethylene evolution from pineapple plants before application of ethephon (**ETLB**). Data are shown for three replications (**REP**).

TREATMENT	REP	LFTS	TPTS	MDTS	LFST	TPST	MDST	ETLB[a]
		- - - - - - - - - % - - - - - - - - -						
N_1	1	12.9	14.6	10.4	2.49	14.9	18.9	0.142
	2	17.6	11.6	10.8	2.07	12.1	14.6	0.128
	3	14.6	13.2	11.1	2.37	13.7	17.1	0.091
N_2	1	11.6	11.6	10.1	2.30	11.9	11.6	0.187
	2	10.8	12.6	11.0	1.69	8.3	12.7	0.364
	3	13.5	14.9	11.6	2.17	19.9	18.0	0.214
N_3	1	11.0	12.9	9.8	1.76	6.8	8.7	0.148
	2	10.2	11.2	10.2	1.72	11.3	14.1	0.255
	3	9.5	14.4	10.8	2.03	15.9	17.8	0.171
N_4	1	7.0	12.5	8.5	1.38	6.9	7.4	0.392
	2	9.8	13.0	8.6	1.47	12.6	15.8	0.264
	3	8.7	13.2	9.0	1.87	10.6	17.1	0.164

[a] Microliter plant^{-1} hour^{-1}

Table 27. Effect of nitrogen (**NITR**) and ethephon (**ETHE**) on ethylene evolution from pineapple plants for five days after ethephon application (**ETL1-5**), percentage of plant flowering (**%FLW**), inflorescence length (**INFL**), peduncle diameter (**PEDD**), and number of florets per inflorescence (**#FLR**). Data are shown for three replications (**REP**).

TREATMENT		REP	ETL1	ETL2	ETL3	ETL4	ETL5	%FLW	INFL	PEDD	#FLR
NITR	ETHE										
	-mg-		- - - - µl plant^{-1} hr^{-1} - - - -						- - mm - -		
N_1	0.5	1	0.251	0.080	0.042	0.025	0.027	85.7	39.6	13.4	84.0
		2	0.603	0.076	0.049	0.044	0.021	66.7	42.4	14.2	96.0
		3	0.307	0.043	0.035	0.022	0.011	57.1	37.3	15.0	98.4
	5.0	1	2.869	2.034	0.070	0.051	0.022	100.0	46.8	14.5	84.0
		2	3.005	1.704	0.061	0.054	0.029	71.4	44.3	14.3	88.0
		3	3.370	2.528	0.252	0.080	0.081	85.7	50.7	16.0	96.0
N_2	0.5	1	0.421	0.117	0.112	0.036	0.027	71.4	33.7	11.3	72.0
		2	0.478	0.115	0.049	0.029	0.024	100.0	39.2	14.0	96.0
		3	0.410	0.103	0.035	0.028	0.021	85.7	38.7	14.9	102.4
	5.0	1	4.554	3.188	0.405	0.094	0.090	100.0	43.3	14.1	86.4
		2	4.280	2.803	0.713	0.131	0.126	100.0	49.6	15.5	101.6
		3	5.555	2.748	0.364	0.073	0.072	100.0	40.4	15.5	105.6
N_3	0.5	1	0.638	0.357	0.140	0.073	0.043	83.3	36.1	12.5	88.0
		2	0.524	0.296	0.056	0.040	0.035	85.7	34.2	12.6	84.0
		3	0.672	0.309	0.063	0.073	0.053	60.0	39.8	15.9	88.0
	5.0	1	3.916	3.682	1.929	0.797	0.378	71.4	46.0	14.0	96.0
		2	5.373	3.572	1.007	0.355	0.228	100.0	36.5	13.2	88.0
		3	5.282	3.792	1.678	0.739	0.515	85.7	39.1	14.3	92.8
N_4	0.5	1	0.512	0.454	0.211	0.102	0.048	71.4	44.3	12.2	74.4
		2	0.695	0.550	0.248	0.094	0.069	57.1	35.1	12.4	92.0
		3	0.455	0.536	0.192	0.087	0.059	33.3	24.1	11.0	100.0
	5.0	1	4.645	4.891	1.720	0.746	0.432	33.3	33.4	9.7	76.0
		2	6.466	4.562	1.248	0.333	0.204	85.7	35.6	11.8	98.4
		3	7.195	3.847	1.762	0.625	0.264	83.3	33.4	11.5	96.8

TABLE 28. Effects of night temperature **(TEMP)** and duration of exposure to night temperature **(DURN)** on leaf moisture content **(LFMC)**, stem moisture content **(SMMC)**, leaf dry weight **(LFDW)**, stem dry weight **(SMDW)**, leaf nitrogen **(LFN)**, titratable acids at 7:00 a.m. **(TAMN)**, titratable acids at 5:00 p.m. **(TAEN)**, leaf starch **(LFST)**, stem (top) starch **(TPST)**, and stem (middle) starch **(MDST)**, leaf total soluble sugars **(LFTS)**, stem (top) total sugars **(TPTS)** of pineapple. Data are shown for three replications **(REP)**.

TREATMENT														
TEMP	DURN	REP	LFMC	SMMC	LFDW	SMDW	LFN	TAMN	TAEN	LFST	TPST	MDST	LFTS	TPTS
			- - % - -		- - g - -		-%-	meq $100g^{-1}$		- - - - - % dry wt. - - - - -				
20	1	1	86.3	85.9	110.0	12.2	1.60	38.2	5.2	2.26	10.62	18.31	13.69	17.7
		2	87.4	86.5	96.4	7.8	1.48	35.2	5.9	3.09	9.38	17.63	12.11	16.5
		3	85.8	87.4	117.8	10.9	1.45	35.2	6.7	3.40	12.29	17.08	14.87	18.5
	2	1	85.9	86.5	117.2	9.7	1.52	36.7	6.0	2.94	11.72	13.25	11.25	17.4
		2	87.0	87.8	113.9	10.3	1.53	38.7	6.1	4.14	10.44	11.15	10.74	15.6
		3	85.6	85.5	125.0	13.2	1.45	38.4	6.0	4.05	15.55	21.50	15.30	19.3
	3	1	86.7	86.8	126.0	11.3	1.51	36.0	4.6	3.60	11.78	10.52	14.23	15.3
		2	86.0	85.9	150.0	13.9	1.68	37.1	7.0	3.03	17.00	16.65	9.61	17.7
		3	84.9	84.4	146.4	18.5	1.42	35.2	5.1	4.30	18.48	24.29	17.27	19.6
25	1	1	87.0	87.8	109.2	10.1	1.45	30.3	6.2	2.95	10.95	14.33	12.94	17.5
		2	87.2	86.9	99.0	8.4	1.55	26.6	7.6	2.51	10.43	12.69	13.50	15.4
		3	85.8	86.5	146.6	14.1	1.57	32.4	7.4	3.04	12.35	14.13	12.09	17.5
	2	1	88.2	86.4	99.9	8.5	1.70	27.6	7.2	2.92	10.01	11.70	10.01	15.2
		2	87.6	86.6	124.2	11.8	1.69	36.3	7.1	3.01	13.56	12.74	10.92	16.9
		3	84.7	85.4	129.4	14.7	1.59	36.2	5.5	3.93	14.07	13.02	13.20	18.8
	3	1	86.6	87.7	121.2	9.9	1.80	30.0	5.8	3.30	8.40	7.70	10.79	14.5
		2	87.4	86.1	111.8	11.2	1.46	28.9	5.9	2.68	11.15	13.72	14.67	16.6
		3	84.6	85.1	162.3	17.0	1.46	33.0	5.7	2.33	14.98	20.51	14.61	16.8

Table 28. Continued.

TREATMENT		REP	LFMC	SMMC	LFDW	SMDW	LFN	TAMN	TAEN	LFST	TPST	MDST	LFTS	TPTS
TEMP	DURN													
			- - % - -		- - g - -		-%-	meq $100g^{-1}$		- - - - - % dry wt. - - - - -				
30	1	1	86.7	87.7	102.6	9.3	1.61	17.6	4.2	2.47	9.63	8.28	10.46	15.0
		2	87.1	88.4	82.1	7.1	1.76	20.6	4.7	2.95	9.44	6.95	7.53	13.3
		3	83.8	82.1	170.2	22.7	1.69	18.1	4.0	1.93	13.92	12.69	10.29	20.0
	2	1	87.6	87.2	97.6	8.1	1.64	17.6	4.4	1.96	8.27	8.81	8.87	14.2
		2	87.4	88.2	100.5	7.7	1.80	20.9	4.7	2.36	7.65	5.18	7.79	13.9
		3	84.4	83.4	113.8	11.6	1.49	18.1	3.4	1.58	8.66	5.11	13.12	17.7
	3	1	87.6	86.8	111.0	11.1	1.80	17.3	4.7	1.98	8.96	5.98	10.56	14.3
		2	86.8	87.0	116.4	10.6	1.98	14.2	5.2	0.96	7.91	6.25	7.56	13.5
		3	84.5	81.4	132.8	17.0	1.61	19.3	4.0	1.28	7.41	5.18	12.47	18.6

TABLE 29. Effects of night temperature **(TEMP)** and duration of exposure to night temperature **(DURN)** on stem (middle) total soluble sugars **(MDTS)**, percentage of flowering **(%FLW)**, inflorescence length **(INFL)**, peduncle diameter **(PEDD)**, number of florets per inflorescence **(#FLR)**, ethylene evolution from the pineapple plants on first to forth days after ethephon application **(ETL1-4)**. Data are shown for three replications **(REP)**.

Treatment		REP	MDTS	%FLW	INFL	PEDD	#FLR	ETL1	ETL2	ETL3	ETL4
TEMP	DURN										
			- % -		- - mm - -			- - - $\mu l\ pl^{-1}\ hr^{-1}$ - - -			
20	1	1	17.5	100.0	56.23	16.08	80.0	3.186	2.696	0.776	0.143
		2	15.1	60.0	46.38	15.37	80.0	2.900	2.900	1.634	0.153
		3	12.7	100.0	101.88	19.86	86.4	4.311	2.223	0.560	0.143
	2	1	16.3	100.0	62.80	17.36	90.4	3.227	2.839	0.654	0.143
		2	14.0	100.0	38.01	14.13	82.4	3.431	1.879	0.715	0.184
		3	13.0	100.0	96.77	20.94	90.4	2.749	2.512	0.521	0.150
	3	1	13.1	100.0	45.57	16.25	85.6	3.758	2.778	1.216	0.184
		2	14.0	100.0	45.77	13.96	82.4	2.696	2.369	0.633	0.092
		3	14.5	100.0	75.16	18.53	84.0	3.111	2.090	0.528	0.132
25	1	1	13.0	100.0	65.65	17.84	90.4	5.042	3.085	0.776	0.111
		2	11.7	100.0	45.56	15.20	82.4	3.840	3.207	0.490	0.112
		3	13.7	100.0	99.62	20.29	80.0	5.221	2.552	0.741	0.191
	2	1	12.0	100.0	62.99	17.19	84.0	4.943	1.634	0.490	0.102
		2	13.3	100.0	63.45	16.23	80.0	3.799	2.900	0.878	0.276
		3	11.8	100.0	74.55	18.45	80.0	8.829	2.933	0.664	0.169
	3	1	12.9	100.0	73.49	17.76	86.4	4.534	2.410	0.674	0.133
		2	12.1	100.0	68.92	17.14	94.4	3.922	2.124	0.731	0.276
		3	13.6	100.0	82.36	19.61	80.0	6.453	3.226	0.881	0.134

Table 29. Continued.

Treatment TEMP	DURN	REP	MDTS	%FLW	INFL	PEDD	#FLR	ETL1	ETL2	ETL3	ETL4
			- % -		- - mm - -			- - - $\mu l\ pl^{-1}\ hr^{-1}$ - - -			
30	1	1	12.2	25.0	81.10	17.14	88.0	6.863	2.410	0.542	0.082
		2	11.6	50.0	59.25	15.36	84.0	7.679	2.778	0.633	0.061
		3	16.7	100.0	83.93	16.72	70.4	9.497	2.904	0.253	0.139
	2	1	10.5	100.0	62.08	15.12	78.4	6.372	2.603	0.296	0.082
		2	11.2	50.0	52.17	15.60	84.0	7.394	3.207	0.327	0.051
		3	12.4	100.0	89.89	18.00	66.4	8.007	3.967	0.711	0.151
	3	1	12.2	75.0	65.07	15.29	80.0	9.354	2.696	0.542	0.143
		2	11.3	75.0	60.02	15.50	80.0	6.944	2.982	0.449	0.051
		3	14.5	100.0	59.24	15.01	58.4	9.884	1.899	0.217	0.158

Table 30. Effects of nitrogen and night temperature on leaf moisture content **(LFMC)**, stem moisture content **(SMMC)**, leaf dry weight **(LFDW)**, stem dry weight **(SMDW)**, leaf chlorophyll **(LFCH)**, titratable acids at 7:00 a.m. **(TAMN)**, titratable acids at 5:00 p.m. **(TAEN)**, ethylene evolution before application of ethephon **(ETLB)**, ethylene evolution from pineapple plants on first to fourth days after application of application **(ETL1-4)**. Data are shown for three replications **(REP)**.

TREATMENT		REP	LFMC	SMMC	LFDW	SMDW	LFCH	TAMN	TAEN	ETLB	ETL1	ETL2	ETL3	ETL4
TEMP	NITR													
			- - % - -		- - g - -		mg g^{-1}	meq $100g^{-1}$		- - - - - µl pl^{-1} hr^{-1} - - - - -				
20	N_1	1	87.5	84.5	103.8	12.9	0.244	27.6	3.5	0.093	2.672	3.724	0.277	0.285
		2	86.1	82.7	122.7	14.9	0.284	31.8	3.5	0.064	4.365	3.239	0.213	0.207
		3	85.9	82.5	140.8	17.0	0.263	29.6	3.9	0.062	2.885	3.265	0.185	0.131
	N_2	1	87.1	83.1	141.2	20.0	0.378	27.8	5.3	0.049	2.659	4.340	0.240	0.541
		2	86.7	84.6	168.0	16.9	0.429	36.5	5.1	0.052	2.183	5.022	0.324	0.507
		3	84.4	80.1	142.7	22.2	0.491	40.1	5.7	0.127	2.634	4.786	0.361	0.586
30	N_1	1	86.3	86.3	127.7	15.5	0.319	16.9	3.2	0.105	6.924	1.246	0.817	0.270
		2	85.6	81.9	107.6	18.4	0.370	22.8	4.9	0.081	7.853	1.914	0.635	0.255
		3	85.6	83.0	111.1	16.8	0.324	23.6	3.7	0.158	8.103	2.544	0.515	0.222
	N_2	1	87.2	85.4	120.2	15.0	0.474	21.5	4.6	0.149	7.100	3.003	0.222	0.578
		2	85.9	85.9	111.4	12.7	0.492	23.2	6.4	0.072	7.250	4.812	0.450	0.563
		3	85.3	84.1	113.8	13.8	0.526	30.1	4.9	0.146	6.147	3.186	0.407	0.515
AMB	N_1	1	87.4	84.4	104.0	13.1	0.208	25.4	2.7	0.046	2.835	2.426	0.308	0.188
		2	86.4	82.5	115.0	16.6	0.232	34.7	3.7	0.053	2.960	3.514	0.166	0.263
		3	85.7	82.1	132.4	21.4	0.268	36.3	4.4	0.050	2.308	2.806	0.416	0.379
	N_2	1	86.0	83.1	117.5	13.2	0.445	25.8	4.6	0.053	4.190	2.504	0.444	0.334
		2	85.9	84.3	106.7	11.8	0.421	35.8	5.5	0.037	6.899	1.770	0.549	0.162
		3	86.1	84.8	133.1	13.9	0.426	32.5	5.3	0.059	4.792	2.990	0.582	0.477

Table 31. Effects of night temperature and nitrogen on leaf nitrogen **(LFN)**, leaf starch **(LFST)**, stem (top) starch **(TPST)**, stem (middle) starch **(MDST)**, leaf total soluble sugars **(LFTS)**, stem (top) total soluble sugars **(TPTS)**, stem (middle) total soluble sugars **(MDTS)**, percentage flowering **(%FLW)**, inflorescence length **(INFL)**, peduncle diameter **(PEDD)**, and number of florets per inflorescence **(#FLR)** of pineapple. Data are shown for three replications **(REP)**.

TREATMENT		REP	LFN	LFST	TPST	MDST	LFTS	TPTS	MDTS	%FLW	INFL	PEDD	#FLR
TEMP	NITR												
			- - - - - - - % dry wt. - - - - - - -								- - mm - -		
20	N_1	1	1.13	3.4	8.5	12.5	15.6	20.9	13.3	100.0	44.2	14.1	85
		2	1.00	4.1	15.1	24.3	16.5	19.4	12.1	100.0	59.4	16.0	77
		3	0.95	4.4	14.2	21.5	17.5	22.3	14.4	100.0	55.4	16.8	82
	N_2	1	2.55	2.0	6.0	16.8	6.1	16.5	11.4	80.0	38.7	14.6	84
		2	2.78	3.8	5.5	16.9	5.2	15.5	11.3	100.0	73.6	17.1	80
		3	2.67	3.2	12.4	20.6	6.2	16.5	10.9	100.0	71.3	17.7	86
30	N_1	1	1.18	1.1	6.5	16.5	11.4	20.0	14.4	40.0	57.9	15.7	72
		2	1.11	2.2	13.3	21.6	11.6	17.4	10.8	100.0	62.1	14.8	64
		3	1.11	2.1	13.5	22.1	12.0	19.5	10.4	100.0	72.6	15.8	67
	N_2	1	2.41	1.6	3.1	7.6	3.2	12.3	10.1	60.0	39.2	12.5	78
		2	2.66	1.9	2.7	11.1	2.7	11.8	8.1	100.0	54.9	13.4	66
		3	2.80	0.7	6.0	12.6	3.3	13.3	10.6	100.0	42.5	12.4	66
AMB	N_1	1	1.16	1.6	6.9	17.3	15.5	20.7	12.5	60.0	42.4	14.3	78
		2	0.92	3.9	11.8	22.2	16.5	17.1	10.9	100.0	65.8	16.6	77
		3	0.98	4.0	14.4	21.4	15.7	18.4	11.7	100.0	57.4	15.6	83
	N_2	1	2.79	1.4	7.1	17.5	4.7	15.4	10.3	60.0	45.7	14.8	88
		2	2.55	3.6	6.9	13.6	5.5	12.9	9.1	100.0	58.8	15.7	80
		3	2.53	2.8	7.2	14.2	5.0	12.2	8.7	100.0	55.6	15.8	78

Table 32. Effect on night temperature (**TEMP**) and nitrogen (**NITR**) on leaf sucrose (**LSUC**), glucose (**LGLU**), fructose, stem sucrose (**LFRU**), stem (top) sucrose (**TSUC**), glucose (**TGLU**), fructose (**TFRU**), stem (middle) sucrose (**MSUC**), glucose (**MGLU**), and fructose (**MFRU**) of pineapple. Data are shown for three replications (**REP**).

TREATMENT		REP	LSUC	LGLU	LFRU	TSUC	TGLU	TFRU	MSUC	MGLU	MFRU
TEMP	NITR										
			- - - - - - - - - - % dry wt. - - - - - - - - - -								
20	N_1	1	2.4	7.2	9.0	8.9	3.2	3.1	3.6	4.1	3.6
		2	2.9	7.5	11.5	8.9	3.9	3.9	3.1	2.6	1.9
		3	2.4	6.7	10.8	10.1	4.7	3.9	3.7	4.0	2.0
	N_2	1	2.7	(.)[a]	3.8	8.1	1.1	1.5	4.1	2.3	2.8
		2	3.3	(.)	2.0	7.8	1.0	1.4	4.0	1.8	1.9
		3	3.6	(.)	4.1	9.6	1.1	1.4	5.1	1.8	1.9
30	N_1	1	1.7	3.6	9.5	9.4	3.3	3.5	4.7	4.2	3.6
		2	1.4	3.4	8.3	7.6	2.9	3.1	3.8	2.6	2.5
		3	0.9	2.7	5.9	6.6	4.0	4.2	3.8	2.2	2.0
	N_2	1	2.9	(.)	(.)	5.9	1.1	(.)	3.4	1.4	1.9
		2	0.7	(.)	(.)	4.9	1.1	(.)	3.2	0.8	(.)
		3	0.5	(.)	(.)	6.1	1.1	(.)	3.3	1.8	1.4
AMB	N_1	1	1.6	5.5	9.5	9.8	2.4	2.6	4.3	3.3	3.2
		2	1.4	6.4	11.0	7.3	2.9	2.9	3.3	2.8	1.8
		3	1.5	4.1	8.3	6.1	2.5	1.6	3.5	3.4	2.0
	N_2	1	3.2	(.)	0.8	9.5	0.9	1.4	4.4	1.9	2.5
		2	2.7	(.)	3.9	6.1	0.3	(.)	3.0	1.4	1.7
		3	2.2	(.)	4.1	5.4	0.5	(.)	2.8	1.3	2.1

[a] None detected.

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